PB87-132833

Development of Preservation Techniques and Establishment of Maximum Holding Times Inorganic Constitutents of the National Pollutant Discharge Elimination System and Safe Drinking Water Act

Environmental Science and Engineering, Inc.
Gainesville, FL

Prepared for

Environmental Monitoring and Support Lab.
Cincinnati, OH

Dec 86

U.S. Department of Commerce
National Technical Information Service
NTIS

PB87-132833

EPA/600/4-86/043
December 1986

DEVELOPMENT OF PRESERVATION TECHNIQUES AND ESTABLISHMENT OF MAXIMUM HOLDING TIMES

Inorganic Constituents of the National Pollutant Discharge Elimination System and Safe Drinking Water Act

by

Hugh S. Prentice, J. Edward Singley,
Louis J. Bilello, James T. McClave, Karen L. Tuttle,
Edward M. Kellar, and Mary G. Schultz

Environmental Science and Engineering, Inc.
P.O. Box ESE
Gainesville, Florida 32602

Contract No. 68-03-3066

Project Officer

Daniel Bender
Office of the Director
Environmental Monitoring and Support Laboratory
Cincinnati, Ohio 45268

Environmental Monitoring and Support Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, Ohio 45268

REPRODUCED BY
U.S. DEPARTMENT OF COMMERCE
NATIONAL TECHNICAL
INFORMATION SERVICE
SPRINGFIELD, VA. 22161

TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA/600/4-86/043	2.	3. RECIPIENT'S ACCESSION NO. PB87 132833/AS
4. TITLE AND SUBTITLE DEVELOPMENT OF PRESERVATION TECHNIQUES & ESTABLISHMENT OF MAXIMUM HOLDING TIMES: Inorganic Constituents of the National Pollutant Discharge Elimination System & Safe Drinking Water Act		5. REPORT DATE December 1986 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) H.S. Prentice, J.E. Singley, L.J. Bilello, J.T. McClave, K. L. Tuttle, E.M. Kellar, and M.G. Schultz		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Environmental Science and Engineering, Inc. P.O. Box ESE Gainesville, Florida 32602		10. PROGRAM ELEMENT NO. CBEBIC 11. CONTRACT/GRANT NO. 68-03-3066
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Monitoring and Support Laboratory U.S. Environmental Protection Agency 26 West St. Clair Street Cincinnati, Ohio 45268		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA 600/6

15. SUPPLEMENTARY NOTES

16. ABSTRACT

This research program was conducted to develop statistically acceptable, experimentally determined maximum holding times (MHT) from quantitative, legally defensible data acquired from representative real-world samples. Ten National Pollutant Discharge Elimination System (NPDES) and two Safe Drinking Water Act (SDWA) compliance parameters were studied in a total of 18 experiments. Experimental results were compared to EPA-proposed, recommended, or required MHT values.

Large volumes of samples were collected from three different water sources and used for the experimental analyses of each study parameter. Each representative large-volume sample was transported to the laboratory and composited within 24 hours for mixing and dispensing of samples for the experiments. Analyses of the dispensed samples began within 24 hours after mixing and within 48 hours of sample collection.

The development of a statistical protocol to specify experimentally determined MHTs included statistical tests to: choose the best model (zero or first order), evaluate the lack of linear fit for the model, and calculate MHTs and the 90-percent lower confidence level of the calculated MHT. The calculated MHT was based on 10 percent concentration changes from the intercept of the best-fit regression line. Except for mercury and cyanide in wastewater, experimentally determined MHTs of all study parameters exceeded either the proposed or required MHT values.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS (This Report) Unclassified	21. NO. OF PAGES 163
	20. SECURITY CLASS (This page) Unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

NOTICE

The information in this document has been funded wholly or in part by the United States Environmental Protection Agency under Contract No. 68-03-3066 to Environmental Science and Engineering, Inc. It has been subject to the Agency's peer and administrative review, and it has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWORD

Environmental measurements are required to determine the quality of the ambient waters and the character of waste effluents. The Environmental Monitoring and Support Laboratory--Cincinnati conducts research to:

1. Develop and evaluate methods to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.
2. Investigate methods for the concentration, recovery, and identification of viruses, bacteria, and other microbiological organisms in water, and determine the responses of aquatic organisms to water quality.
3. Develop and operate an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.
4. Develop and operate a computerized system for instrument automation leading to improved data collection, analysis, and quality control.

This research project was designed to determine the optimal length of time a sample could be stored using accepted preservation techniques prior to analysis for certain pollutants.

Robert L. Booth, Director
Environmental Monitoring and Support
Laboratory--Cincinnati

ABSTRACT

Statistically defined, experimentally derived maximum holding times (MHT) were determined from data acquired using accepted preservation and storage techniques on spiked representative real-world samples. Fifteen experiments were conducted for 10 National Pollutant Discharge Elimination System (NPDES) compliance parameters and three for two Safe Drinking Water Act (SDWA) compliance parameters. Three different water or wastewater sources were evaluated for each experiment. The NPDES parameters were phenols, cyanide, mercury, ammonia, nitrate plus nitrite, fluoride, total Kjeldahl nitrogen, total phosphorus, total organic carbon, and sulfide. Multiple experiments were performed to evaluate MHT's for different preservation techniques for phenols and mercury. The SDWA parameters were nitrate and fluoride, with two experiments performed to evaluate MHT's for different preservation techniques for nitrate. Experimental results were compared to United States Environmental Protection Agency (EPA)-proposed, recommended, or required MHT values.

The protocol to specify experimentally determined MHT's included statistical tests to: choose the best kinetic model (zero or first order); evaluate the lack of linear fit for the model; and calculate a critical time (CT) and the 90 percent lower confidence limit (LCL) of the CT. The CT was based on 10 percent concentration changes from the intercept of the best-fit regression line. The experimentally determined MHT's were specified as either the 90 percent LCL of the CT or the maximum time of the designed experiment, when the LCL was longer.

Except for mercury and cyanide in wastewater, experimentally determined MHT's of all study analytes exceeded the proposed or required MHT values.

This report was submitted in fulfillment of Contract No. 68-03-3066 by Environmental Science and Engineering, Inc. (ESE), under sponsorship of the United States Environmental Protection Agency. The study was conducted from May 1981 to June 1982.

CONTENTS

Disclaimer ii
Foreword iii
Abstract iv
Figures. vii
Tables viii
Acknowledgments. x

1.0 Introduction 1
  1.1 Background. 1
  1.2 Project Objective and Approach. 4
  1.3 Objective of this Report. 4
2.0 Conclusions. 7
3.0 Recommendations. 9
4.0 Experimental Design and Methodology. 11
  4.1 Experimental Design 11
  4.2 Methodology 11
    4.2.1 Site selection. 13
    4.2.2 Data management and sample custody. 13
    4.2.3 Sampling and transport. 22
    4.2.4 Sample receiving, preparation for analysis, and storage 29
    4.2.5 Sample analysis 36
    4.2.6 Quality assurance 36
    4.2.7 Protocol for statistical tests and logic 40
5.0 Results and Discussion 45
  5.1 Phenols 53
    5.1.1 Experiments and Results 53
    5.1.2 Statistical Evaluation. 55
  5.2 Cyanide 64
    5.2.1 Experiments and Results 64
    5.2.2 Statistical Evaluation. 65
    5.2.3 Discussion. 66
  5.3 Mercury 70
    5.3.1 Experiments and Results 70
    5.3.2 Statistical Evaluation. 72
    5.3.3 Discussion. 73
  5.4 Nitrate and Nitrate Plus Nitrite. 84
    5.4.1 Experiments and Results 84
    5.4.2 Statistical Evaluation. 86
  5.5 Fluoride. 95
    5.5.1 Experiments and Results 95
    5.5.2 Statistical Evaluation. 96

CONTENTS

5.6 Ammonia 103
    5.6.1 Experiments and Results 103
    5.6.2 Statistical Evaluation. 103
5.7 Total Kjeldahl Nitrogen 108
    5.7.1 Experiments and Results 108
    5.7.2 Statistical Evaluation. 108
5.8 Total Organic Carbon. 113
    5.8.1 Experiments and Results 113
    5.8.2 Statistical Evaluation. 113
5.9 Total Phosphorus. 118
    5.9.1 Experiments and Results 118
    5.9.2 Statistical Evaluation. 118
5.10 Sulfide 123
    5.10.1 Experiments and Results 123
    5.10.2 Statistical Evaluation. 123

References 128
Appendix 130

Statistical Program Documentation 130

FIGURES

Number Page

1-1 Graphic representation of MHT definition 5
4-1 Experimental detail for a time-sequenced analysis of a parameter in a single matrix. 12
4-2 Site selection process 14
4-3 Example logsheet used for field samples. 18
4-4 Example chain-of-custody form. 19
4-5 Example labels 20
4-6 Example logsheet for file (field group) set up for each experiment. 21
4-7 Example memorandum for notification of sample lots for analysis 23
4-8 Example sample lot (batch) form for reporting analysis and QC information. 24
4-9 Example field sampling plan. 26
4-10 Mixing device for homogenized sample 32
4-11 Example homogeneity documentation form 33
4-12 Flow chart summarizing statistical protocol. 41
5-1 Statistical results of Experiment 1 for phenols. 61
5-2 Statistical results of Experiment 2 for phenols. 62
5-3 Statistical results of Experiment 3 for phenols. 63
5-4 Statistical results of Experiment 4 for cyanide. 69
5-5 Statistical results of Experiment 5 for mercury. 80
5-6 Statistical results of Experiment 6 for mercury. 81
5-7 Statistical results of Experiment 7 for mercury. 82
5-8 Statistical results of Experiment 8 for mercury. 83
5-9 Statistical results of Experiment 9 for nitrate. 92
5-10 Statistical results of Experiment 10 for nitrate 93
5-11 Statistical results of Experiment 15 for nitrate 94
5-12 Statistical results of Experiment 11 for fluoride. . . .101
5-13 Statistical results of Experiment 13 for fluoride. . . .102
5-14 Statistical results of Experiment 12 for ammonia 107
5-15 Statistical results of Experiment 14 for TKN 112
5-16 Statistical results of Experiment 16 for TOC 117
5-17 Statistical results of Experiment 17 for phosphorus. . .122
5-18 Statistical results of Experiment 18 for sulfide 127

TABLES

Number Page

1-1 Experiments Required to Investigate Recommended Holding Times 2
4-1 Primary SIC Codes of Interest and Parameters Permitted. . 15
4-2 Final List of SIC Code Industrial Effluents Sampled for Each Parameter and Matrix 17
4-3 Field Sample Volumes Required for Each Experiment 28
4-4 Checklist of Containers and Glassware Needed. 30
4-5 Sample Volume Required for Each Experiment. 31
4-6 Preservation Techniques for Each Experiment 34
4-7 Constitution of Matrix Spikes 35
4-8 Analytical Methods. 37
5-1 Summary of Experimentally Determined MHTs 46
5-2 Summary of Approximate Concentration Levels (mg/L) Studied for Each Sample Matrix. 48
5-3 Summary of Percent Standard Deviations (CV) Derived from the Unexplained Variability in the Lack of Linear Fit ANOVA Test 49
5-4 Phenols Results for Samples Analyzed as Time = 0. 57
5-5 Phenols Raw Data and Summary Statistics for Experiment 1. 58
5-6 Phenols Raw Data and Summary Statistics for Experiment 2. 59
5-7 Phenols Raw Data and Summary Statistics for Experiment 3. 60
5-8 Cyanide Results for Samples Analyzed at Time = 0. 67
5-9 Cyanide Raw Data and Summary Statistics for Experiment 4. 68
5-10 Mercury Results for Samples Analyzed at Time = 0. 75
5-11 Mercury Raw Data and Summary Statistics for Experiment 5. 76
5-12 Mercury Raw Data and Summary Statistics for Experiment 6. 77
5-13 Mercury Raw Data and Summary Statistics for Experiment 7. 78
5-14 Mercury Raw Data and Summary Statistics for Experiment 8. 79
5-15 Nitrate and Nitrate Plus Nitrite Results for Samples Analyzed at Time = 0. 88

TABLES

Number Page

5-16 Nitrate Raw Data and Summary Statistics for Experiment 9. 89
5-17 Nitrate Raw Data and Summary Statistics for Experiment 10 90
5-18 Nitrate Plus Nitrite Raw Data and Summary Statistics for Experiment 15 91
5-19 Fluoride Results for Samples Analyzed at Time = 0 98
5-20 Fluoride Raw Data and Summary Statistics for Experiment 11 99
5-21 Fluoride Raw Data and Summary Statistics for Experiment 13 100
5-22 Ammonia Results for Samples Analyzed at Time = 0 105
5-23 Ammonia Raw Data and Summary Statistics for Experiment 12 106
5-24 TKN Results for Samples Analyzed at Time = 0 110
5-25 TKN Raw Data and Summary Statistics for Experiment 14 . . 111
5-26 Total Organic Carbon Results for Samples Analyzed at Time = 0 115
5-27 TOC Raw Data and Summary Statistics for Experiment 16 . . 116
5-28 Total Phosphorus Results for Samples Analyzed at Time = 0. 120
5-29 Total Phosphorus Raw Data and Summary Statistics for Experiment 17 121
5-30 Sulfide Results for Samples Analyzed at Time = 0. 125
5-31 Sulfide Raw Data and Summary Statistics for Experiment 18 126

ACKNOWLEDGMENTS

The Project Officer, Dr. Daniel F. Bender of the Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency in Cincinnati, provided valuable guidance. Dr. John A. Glaser and Mr. Stephen A. Quave, also with EPA-Ci, were responsible for developing the original experimental protocol for the definition and procedures used to determine maximum holding times. The cooperation of the various industries and water treatment plants that allowed access for sample collection is greatly appreciated. ESE is particularly indebted to the following people, without whose assistance all the various aspects of the project could not have been completed:

Suzanne Albrecht
Bill Beckwith
Kelly Bergdoll
Pamela Dickinson
Heidi Dunn
Norberto Fernandez
Curtis Fisher
Ernest Frey
Michael Keirn
Evelyn Knauft
Deborah Laisure
Lee Kidd
Angela Moore
John Mousa
William Murry
Guy Newman
Virginia O'Brien
Dwight Roberts
Jeff Shamis
John Smehil
Bruce Smith
Mark Travis
Barbara Weeks
Brenda Weems
Jeffrey West
Rick Wilson
Carol Woodyard

SECTION 1.0

INTRODUCTION

1.1 BACKGROUND

Since 1972, the United States Congress has passed three acts to establish industrial effluent guidelines and to establish maximum contaminant levels (MCL's) in potable waters. As a result of the 1972 Federal Water Pollution Control Act (FWPAC) and the 1977 Clean Water Act, Congress mandated the United States Environmental Protection Agency (EPA) to establish limitations on the amount of pollutants discharged in industrial effluents. The 1975 Safe Drinking Water Act (SDWA) required EPA to establish MCL's in finished potable water for community and private water supplies serving more than 25 connections. Each of these laws requires EPA to enforce its regulations and, concomitantly, to establish procedures for compliance monitoring. Sample collection and analysis procedures have been established by EPA (1979a).

Since compliance monitoring carries with it the potential for legal action, EPA published "Guidelines Establishing Test Procedures for the Analysis of Pollutants; Proposed Regulations" (EPA, 1979b) and corrections to the proposed regulations (EPA, 1979c). These guidelines included a table of acceptable containers, sample preservation techniques, and maximum holding times (MHT's) to be used for samples subjected to analysis to determine compliance with the National Pollutant Discharge Elimination System (NPDES).

For the results of compliance monitoring to be acceptable in litigation as an enforcement tool, the sample preservation techniques and holding times used for monitoring must be scientifically, statistically, and legally defensible. Data must be available to ensure that these required techniques and holding times provide statistically indistinguishable data, within certain limits, from those of a fresh sample.

In some cases, the scientific literature referenced in either the Federal Register (EPA, 1979b and 1979c) or in the "Methods for Chemical Analysis of Water and Waste" (EPA, 1979a) contains insufficient defensible data to legally justify the holding times specified. In fact, some holding time values for various analytes are different in these two documents (see Table 1-1), because they were derived from a variety of literature sources. To resolve these differences, EPA contracted Environmental Science and Engineering, Inc. (ESE) to perform the study described in this document.

TABLE 1-1. EXPERIMENTS REQUIRED TO INVESTIGATE RECOMMENDED HOLDING TIMES

Experiment number	Parameter	Preservation technique	Literature-recommended MHT (days) EPA 1979a	Literature-recommended MHT (days) EPA 1979c	Time factor (t_x) for time-sequenced analysis* (days)	Container [plastic (P) glass (G)]
Wastewater parameters						
1	Phenols	Cool 4°C H_2SO_4 pH<2	—	28	2	P
2	Phenols	Cool 4°C 1 g/L $CuSO_4$ H_3PO_4 pH<4	1	—	1	G
3	Phenols	Cool 4°C H_2SO_4 pH<2	—	28	2	G
4	Cyanide	Cool 4°C NaOH pH>12	1	14	2	P
5	Mercury	HNO_3 pH<2 0.05% $K_2Cr_2O_7$	—	28	2	P
6	Mercury	HNO_3 pH<2 0.05% $K_2Cr_2O_7$	—	28	2	G
7	Mercury	HNO_3 pH<2	38	—	2	G
8	Mercury	HNO_3 pH<2	13	—	1	P
12	Ammonia	Cool 4°C Conc. H_2SO_4 pH<2	1	28	2	P
13	Fluoride	None	7	28	2	P
14	TKN	Cool 4°C Conc. H_2SO_4 pH<2	1	28	2	P
15	Nitrate plus nitrite	Cool 4°C Conc. H_2SO_4 pH<2	1	28	2	P
16	Organic carbon	Cool 4°C Conc. H_2SO_4 pH<2	1	28	2	P

(continued)

TABLE 1-1. (continued)

Experiment number	Parameter	Preservation technique	Literature-recommended MHT (days)		Time factor (tx) for time-sequenced analysis* (days)	Container [plastic (P) glass (G)]
			EPA 1979a	EPA 1979c		
17	Total phosphorus	Cool 4°C Conc. H_2SO_4 pH<2	1	28	2	P
18	Sulfide	Cool 4°C 2 ml per liter of 2N zinc acetate	1	28	2	P
Drinking water parameters			EPA 1978			
9	Nitrate	Conc. H_2SO_4 pH<2	14		1	P
10	Nitrate	Cool 4°C	2		1	P
11	Fluoride	None	30		2	P

* Time-sequenced analysis occurred at the following intervals: 0 tx, 1 tx, 2 tx, 4 tx, 8 tx, and 16 tx.

°C = Degrees Celsius.

1.2 PROJECT OBJECTIVE AND APPROACH

The objective of this study was to produce statistically defined, experimentally determined MHT's using data acquired from representative real-world samples. The data were used to calculate MHT's according to a mathematically acceptable definition for 10 NPDES and two SDWA compliance analytes. Three different wastewater matrices from Standard Industry Classification (SIC) code industries that produce effluents most likely to contain the specified parameters were required for each parameter. Finished water from three potable water treatment plants treating different raw waters using different methods was required for SDWA parameters. For wastewater matrices, cyanide, phenol, mercury, organic carbon, ammonia, fluoride, total phosphorus, nitrate plus nitrite, sulfide, and total Kjeldahl nitrogen (TKN) were studied; for drinking water matrices, nitrate and fluoride were studied. The 18 experiments to determine MHT's for the various analytes and preservation techniques are summarized in Table 1-1.

The time-sequenced experimental data from each experiment were used to calculate experimentally determined MHT's according to a mathematically acceptable definition (see Appendix). The experimentally determined or calculated MHT was defined as the 90 percent lower confidence limit (LCL) of a specified critical time (see Figure 1-1). The critical time (CT) was defined as the time at which a 10 percent change in the analyte concentration (the intercept concentration at time zero) occurred and when the precision of the method allowed that 10 percent change to be a statistically significant difference at the 90 percent confidence level. When the precision of the method was not sufficient to discriminate a 10 percent change in the analyte concentration, the CT was that time at which the percent change (greater than 10 percent) in the analyte concentration represented a statistically significant difference at the 90-percent confidence level. An upper limit of a 15 percent change in the analyte concentration was set for determining a valid CT. The experimentally determined MHT's were specified as either the 90 percent LCL of the CT or the maximum time of the designed experiment when the LCL was longer.

Only one of the approved methods of analysis for each analyte was used to provide data to calculate experimentally determined MHT's. It is possible that different analytical methods for an analyte would yield different experimentally determined MHT's based on different precision data. However, approved analytical methods in the Federal Register were specified since they should yield similar data quality. The purpose of this study was to evaluate MHT's in relation to preservation techniques, not in relation to various approved analytical methods.

1.3 OBJECTIVE OF THIS REPORT

The objective of this report is to present the results and discussion of all experiments conducted, including complete references and experimental documentation. This report provides support information to EPA concerning the establishment of the proposed holding times (EPA, 1979b and 1979c).

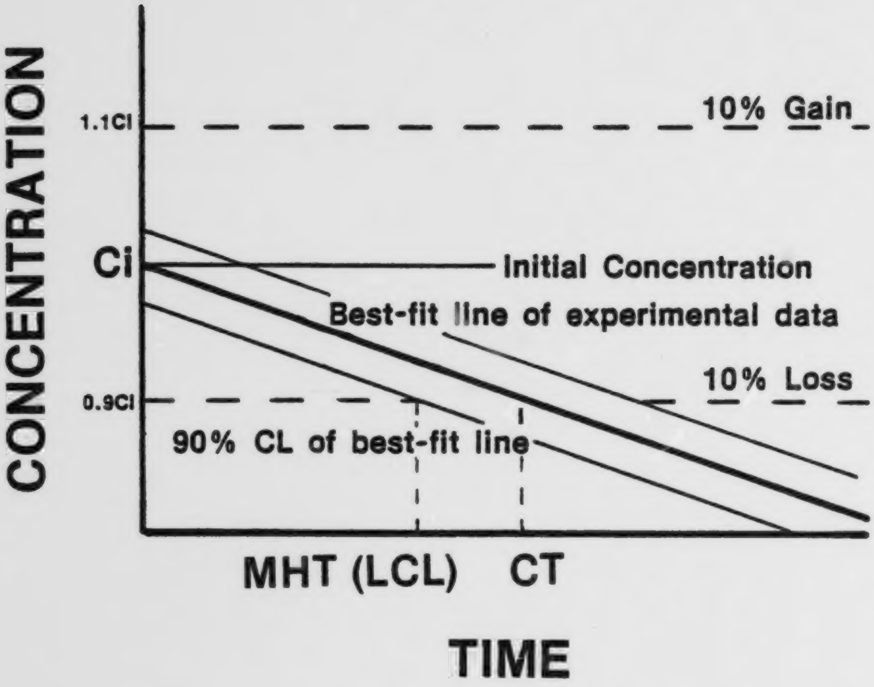


Figure 1-1 GRAPHIC REPRESENTATION OF MHT DEFINITION

Observations concerning the effect of discharge sources and preservation choice on experimentally determined MHT's are also given.

SECTION 2.0

CONCLUSIONS

From the results obtained under the set of conditions used for this study, the following conclusions are drawn:

1. The experimentally determined MHT's were longer than the proposed holding times specified in the Federal Register (EPA, 1979c) and the recommended values from EPA (1979a) for the analysis of the parameters in wastewater samples listed below.

Phenols	Nitrate plus nitrite
Ammonia	Total organic carbon
Fluoride	Total phosphorus
TKN	Sulfide

   For mercury and cyanide, the experimentally determined MHT's were not longer than the proposed or recommended values.
2. The experimentally determined MHT's were longer than the EPA (1978)-required holding time values for the analysis of nitrate and fluoride in samples from finished drinking water.
3. Cyanide concentrations in wastewater samples containing sulfur compounds, which are converted to sulfide during the distillation procedure, cannot accurately be determined using the currently approved analytical method for cyanide (Federal Register, EPA, 1979b) (see Recommendation 1).
4. The major reasons that some experimentally determined MHT's were lower than the proposed and recommended values were: the known variability for mercury analyses, especially at sample concentrations less than 2.0 micrograms per liter (ug/L), and the possible effectiveness of 0.05 percent potassium dichromate ($K_2Cr_2O_7$) in samples acidified with nitric acid (HNO_3) to extract mercury from the walls of new, HNO_3-rinsed polyethylene Hedwin Cubitainers®. The slopes of the best-fit regression lines were not statistically similar for the two experiments using polyethylene Cubitainers®. Decreasing concentrations with time were observed when samples were preserved with HNO_3, except at the highest mercury concentration where no statistical change with time was observed. Increasing concentrations with time were observed when 0.05 percent $K_2Cr_2O_7$ and HNO_3 were used as preservative (see Recommendations 2 and 3).
5. Different sample preservation techniques were studied for nitrate, phenols, and mercury analyses. The two preservation techniques for finished drinking water for nitrate analysis yielded the same experimentally determined MHT's. Of the three preservation experiments for phenols in wastewater, the two in which H_2SO_4 was added to samples stored in plastic or glass provided the longest

SECTION 2.0

CONCLUSIONS

From the results obtained under the set of conditions used for this study, the following conclusions are drawn:

1. The experimentally determined MHT's were longer than the proposed holding times specified in the Federal Register (EPA, 1979c) and the recommended values from EPA (1979a) for the analysis of the parameters in wastewater samples listed below.

Phenols	Nitrate plus nitrite
Ammonia	Total organic carbon
Fluoride	Total phosphorus
TKN	Sulfide

   For mercury and cyanide, the experimentally determined MHT's were not longer than the proposed or recommended values.
2. The experimentally determined MHT's were longer than the EPA (1978)-required holding time values for the analysis of nitrate and fluoride in samples from finished drinking water.
3. Cyanide concentrations in wastewater samples containing sulfur compounds, which are converted to sulfide during the distillation procedure, cannot accurately be determined using the currently approved analytical method for cyanide (Federal Register, EPA, 1979b) (see Recommendation 1).
4. The major reasons that some experimentally determined MHT's were lower than the proposed and recommended values were: the known variability for mercury analyses, especially at sample concentrations less than 2.0 micrograms per liter (ug/L), and the possible effectiveness of 0.05 percent potassium dichromate ($K_2Cr_2O_7$) in samples acidified with nitric acid (HNO_3) to extract mercury from the walls of new, HNO_3-rinsed polyethylene Hedwin Cubitainers®. The slopes of the best-fit regression lines were not statistically similar for the two experiments using polyethylene Cubitainers®. Decreasing concentrations with time were observed when samples were preserved with HNO_3, except at the highest mercury concentration where no statistical change with time was observed. Increasing concentrations with time were observed when 0.05 percent $K_2Cr_2O_7$ and HNO_3 were used as preservative (see Recommendations 2 and 3).
5. Different sample preservation techniques were studied for nitrate, phenols, and mercury analyses. The two preservation techniques for finished drinking water for nitrate analysis yielded the same experimentally determined MHT's. Of the three preservation experiments for phenols in wastewater, the two in which H_2SO_4 was added to samples stored in plastic or glass provided the longest

experimentally determined MHT's. Of the four mercury-in-wastewater preservation experiments, HNO_3 and $K_2Cr_2O_7$ added to samples stored in glass provided the best experimentally determined MHT's.

6. The phenols experiments that used H_2SO_4 as the preservative consistently exhibited higher coefficients of variation for the unexplained variability from regression diagnostics compared to the copper sulfate/phosphoric acid preservation experiments. The highest CV's were from Sample Matrix 3, which also had the highest suspended solids content of the sample matrices collected for the phenols experiments. Since the higher variability is due to the high suspended solids content, the higher CV's were accepted as reasonable for analytical variability, therefore allowing the calculated MHT to be considered valid for that matrix (see Recommendation 5).
7. A statistically significant lack of linear fit was encountered for the experimental data due primarily to larger day-to-day analytical variability compared to within-day analytical variability. This phenomenon is not entirely unexpected and it has caused some concern from a purely statistical standpoint. Specifically, the concern arises from deriving a precise procedure to evaluate when the experimental data exhibits a true lack of linear fit. A true lack of linear fit for the experimental data implies that MHT cannot be validly calculated. The procedure used for this study represents a reasonable approach for the determination of MHT's (see Recommendation 7).

SECTION 3.0

RECOMMENDATIONS

From the results and conclusions of this study, the following are recommended:

1. Experimentally valid MHT's should be determined using EPA's revised analytical method which includes an optional distillation procedure for the analysis of cyanide in the presence of sulfur compounds (EPA, 1983) (see Conclusion 3).
2. Alternative analytical methods for mercury analysis which provide better day-to-day precision than the currently approved method should be developed. A method using sodium borohydride without digestion which was developed by Perkin Elmer (1981) could be considered (see Conclusion 4).
3. The use of polyethylene containers for storing wastewater samples collected for mercury analysis which are preserved with HNO_3 to a pH of less than 2 and 0.05 percent $K_2Cr_2O_7$ should be evaluated further. Future MHT experiments for this preservation protocol should include submitting preserved blanks to the time-sequenced study to verify that the containers used for the study did not contribute to mercury concentrations measured (see Conclusion 4).
4. Additional types of wastewater matrices to ensure the applicability of the proposed and recommended MHT's to all types of wastewater could be evaluated. However, MHT's are clearly matrix dependent. EPA's conservative approach was to cite the shortest MHT and leave the proof of longer MHT's to the user.
5. Conducting additional MHT matrix experiments should be considered. Three different wastewater sample matrices for each study parameter were studied. Several anomalies attributable to the matrix were observed for cyanide, mercury, and phenols (more variability was observed for one phenolics matrix compared to the other two matrices).
6. Actual MHT's could be determined for those parameters which had experimentally determined MHT's exceeding 32 days. Samples sometimes need reanalysis several months after collection to confirm results.
7. Further holding time studies should recognize the problems encountered during this study and possibly incorporate the following two recommendations. First, studies should provide a mechanism to precisely define project-specific analytical

variability for comparison to lack-of-fit variability. Second, future studies should use an analysis of the residuals from the linear regression line to detect outliers and determine the influence from extreme observations (see Conclusion 7).

SECTION 4.0

EXPERIMENTAL DESIGN AND METHODOLOGY

The methodology used to implement the experimental design (see Appendix) was developed to create an accurate written verified record which could be used to trace the samples from the collection sites through the statistical evaluation of the experimental data.

4.1 EXPERIMENTAL DESIGN

Based on the experimental design, three drinking water matrices were sampled for three experiments, and three wastewater matrices were sampled for each parameter of 15 experiments (see Table 1-1). For wastewater or drinking water parameters with multiple experiments (e.g., phenols and mercury in wastewater and nitrate in drinking water), the same sample matrices were collected at the same site, and mixing and analyses were started on the same day. Figure 4-1 summarizes the experimental data for a time-sequenced analysis of one parameter in one matrix. This process was performed 54 times during the project (three matrices times 18 experiments). The protocol for preparing samples for time-sequenced analysis included:

1. Collecting grab samples and shipping to the laboratory within 24 hours;
2. Compositing and homogenizing the grab samples in a large-volume mixing container;
3. Collecting three aliquots for analysis from the composite prior to addition of preservative;
4. Collecting three aliquots for analysis from the composite that contained the preservative;
5. Adding the study analyte to the preserved composite and mixing;
6. Collecting 100 aliquots (dispensed to ensure a large enough population for representative random sampling) from the preserved composite;
7. Selecting three fixed aliquots (Aliquots 1, 50, and 100) for analysis on Day 0 to verify that aliquots were homogeneous; and
8. Randomly selecting 15 aliquots from the remaining 97 aliquots for time-sequenced analysis.

4.2 METHODOLOGY

This section presents the methodology in the following format:

4.2.1 Site Selection
4.2.2 Data Management and Sample Custody
4.2.3 Sampling and Transport
4.2.4 Sample Receiving, Preparation for Analysis, and Storage
4.2.5 Sample Analysis

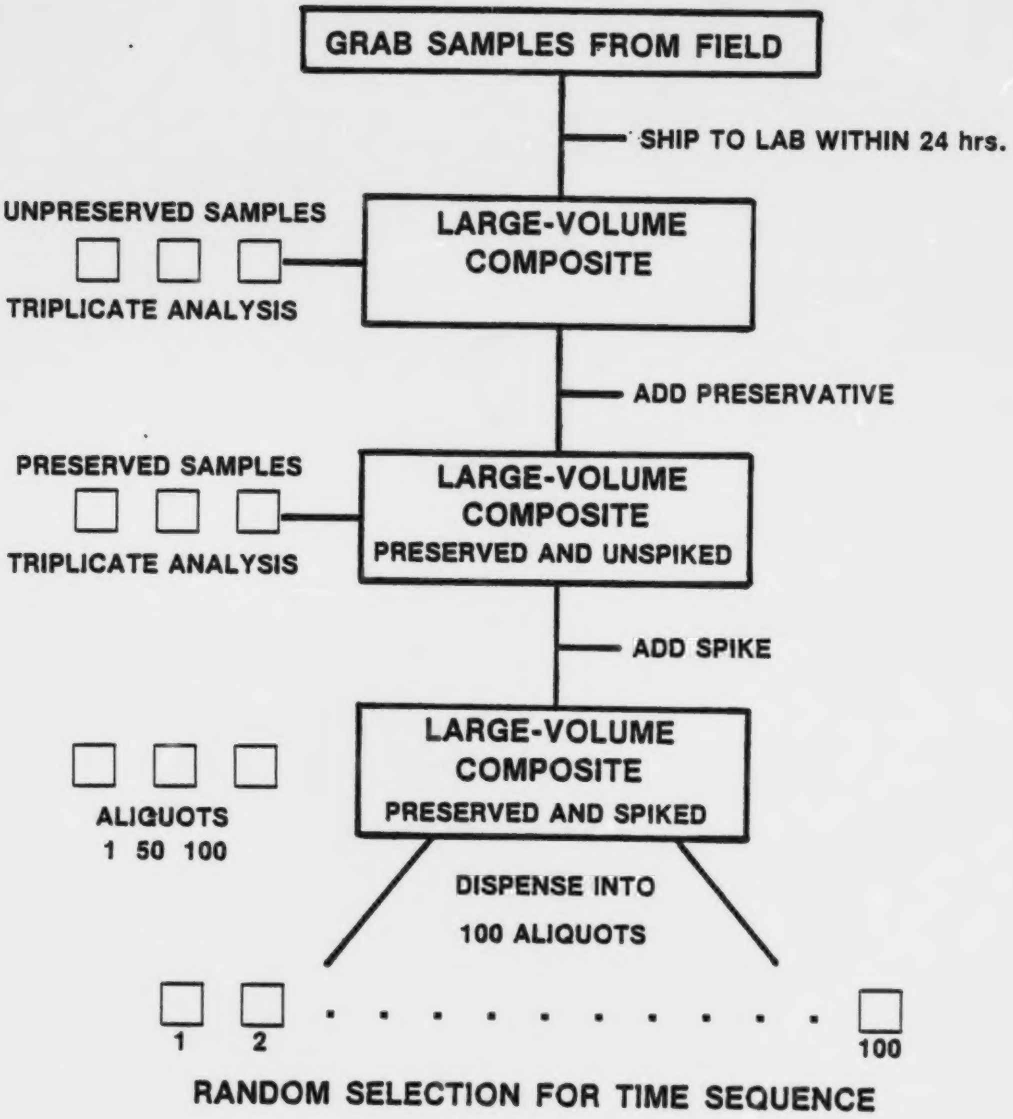

GRAB SAMPLES FROM FIELD
SHIP TO LAB WITHIN 24 hrs.
UNPRESERVED SAMPLES
TRIPLICATE ANALYSIS
LARGE-VOLUME COMPOSITE
ADD PRESERVATIVE
PRESERVED SAMPLES
TRIPLICATE ANALYSIS
LARGE-VOLUME COMPOSITE PRESERVED AND UNSPIKED
ADD SPIKE
ALIQUOTS
1 50 100
LARGE-VOLUME COMPOSITE PRESERVED AND SPIKED
DISPENSE INTO 100 ALIQUOTS
1
2
100
RANDOM SELECTION FOR TIME SEQUENCE

Figure 4-1 EXPERIMENTAL DETAIL FOR A TIME-SEQUENCED ANALYSIS OF A PARAMETER IN A SINGLE MATRIX

4.2.1 Site Selection

Figure 4-2 summarizes the selection of appropriate sampling sites. Initially, 21 industrial SIC codes were chosen from a report provided by EPA (Moore, 1980). These sites were likely to contain the 10 specified parameters (see Table 4-1). EPA Permit Compliance System (PCS) computer files were accessed from the EPA Office of Water Enforcement and Permits, Compliance Branch, Information Management and Evaluation Sections. These PCS printouts were used to choose preliminary candidate sites from EPA Regions IV, V, VI, and VII. Criteria for the selection were plant location respective to ESE's laboratory in Gainesville, Florida, (for timely sample shipment within 24 hours) and permit monitoring requirements for the study parameters.

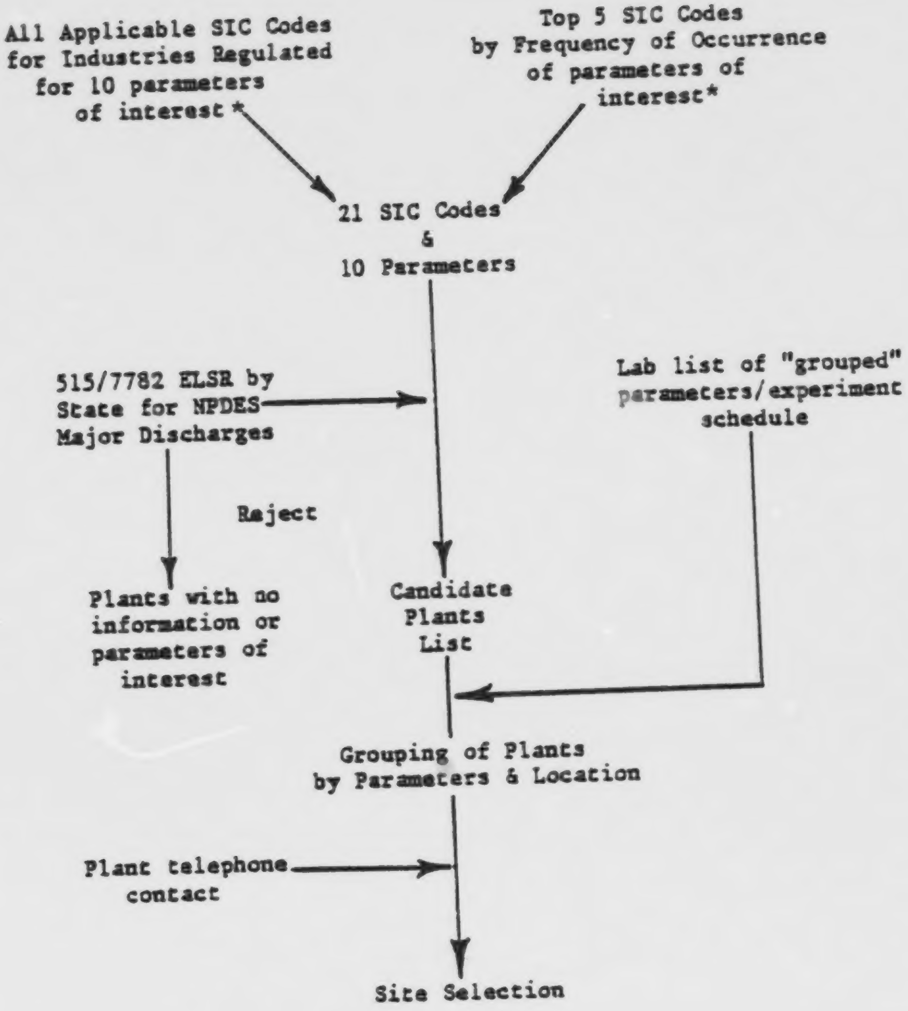
EPA regions were subsequently contacted to obtain complete permit files and monitoring reports for the preliminary candidate sites. The companies were then contacted by telephone and letter to verify wastestream identification monitoring data and permit requirements and to request permission for sample collection. The companies were assured of confidentiality. During the course of the project, some changes from the preliminary candidate sites had to be made for various reasons such as outdated information or shutdown of processes which generated the study parameter.

Table 4-2 is the final list of the SIC-code industrial effluents sampled for each parameter. Drinking water sites were chosen from a selection of water treatment facilities in Florida, because Florida has a wide variety of raw water sources and treatment processes.

4.2.2 Data Management and Sample Custody

The computerized laboratory data management system (Gillespie, 1982) was an integral part of the data management and sample custody procedures. In conjunction with the system, 21 field group computer files were established to control labeling and sample grouping for the 18 experiments. One file was established to assign labels for collected field sample fractions and the 100 dispensed spiked sample aliquots. Fifty-four sample numbers were assigned for each sample collected for the 18 experiments (three matrices for each experiment). Station identification codes for each sample number followed the sequence E1M1, E1M2, E1M3, E2M1...E18M3, etc., where E_ represented the experiment number and M_ the matrix number. Figures 4-3, 4-4, and 4-5 are example field sampling forms and labels (logsheet, chain-of-custody, and labels, respectively).

Eighteen field group computer files were designed to control actual sample analysis and reporting for samples undergoing the time-sequenced analysis of a parameter in a single matrix. Each file was assigned 90 sample numbers, 30 for each of the three matrices for each experiment. Twenty-four samples of the 30 for each matrix [six were quality assurance (QA) reference samples, one for each day of analysis] are identified in Figure 4-1. These 30 samples were logged in after mixing using a logsheet similar to Figure 4-6.


All Applicable SIC Codes for Industries Regulated for 10 parameters of interest *
Top 5 SIC Codes by Frequency of Occurrence of parameters of interest*
21 SIC Codes & 10 Parameters
515/7782 ELSR by State for NPDES Major Discharges
Lab list of "grouped" parameters/experiment schedule
Reject
Plants with no information or parameters of interest
Candidate Plants List
Grouping of Plants by Parameters & Location
Plant telephone contact
Site Selection

* See Tables 2 and 6 in Moore (1980).

Figure 4-2. SITE SELECTION PROCESS

TABLE 4-1. PRIMARY SIC CODES OF INTEREST AND PARAMETERS PERMITTED

SIC code	Description	Parameters covered*									
		P	CN	Hg	NH_3	F	TKN	NO_2+NO_3	TOC	TP	S
0259	Poultry and Eggs, NEC†						X				
1475	Phosphate Rock					X				X	
2211	Broad Woven Fabric Mills, Cotton	X									
2281	Yarn Spinning Mills: Cotton, Manmade Fibers, and Silk										X
2812	Alkalies and Chlorine			X							
2819	Industrial Inorganic Chemicals, NEC				X		X	X			X
2834	Pharmaceutical Preparations							X			
2869	Industrial Organic Chemicals, NEC	X			X		X	X			
2873	Nitrogenous Fertilizers						X	X	X		
2879	Pesticides and Agricultural Chemicals, NEC	X							X		
2911	Petroleum Refining	X			X				X		X
3111	Leather Tanning and Finishing										X
3211	Flat Glass									X	
3312	Blast Furnaces, Steel Works, and Rolling Mills	X	X		X					X	3334
	Primary Production of Aluminum					X					3339
	Primary Smelting and Refining of Nonferrous Metals, NEC			X		X		X			3471
	Electroplating, Plating, Polishing, Anodizing, and Coloring		X								

(Continued)

TABLE 4-1. (continued)

SIC code	Description	Parameters covered*									
		P	CN	Hg	NH_3	F	TKN	NO_2+NO_3	TOC	TP	S
3479	Coating, Engraving, and Allied Services, NEC		X								
3692	Primary Batteries, Dry and Wet			X							
3694	Electrical Equipment for Internal Combustion Engines		X								
4911	Electrical Services			X							X

*NOTE: P = Phenols.
CN = Cyanide.
Hg = Mercury.
NH_3 = Ammonia.
F = Fluoride.
TKN = Total Kjeldahl nitrogen.
NO_2 + NO_3 = Nitrate plus nitrite.
TOC = Total organic carbon.
TP = Total phosphorus.
S = Sulfide.
†NEC = Not Elsewhere Classified.

Source: Moore, 1980.

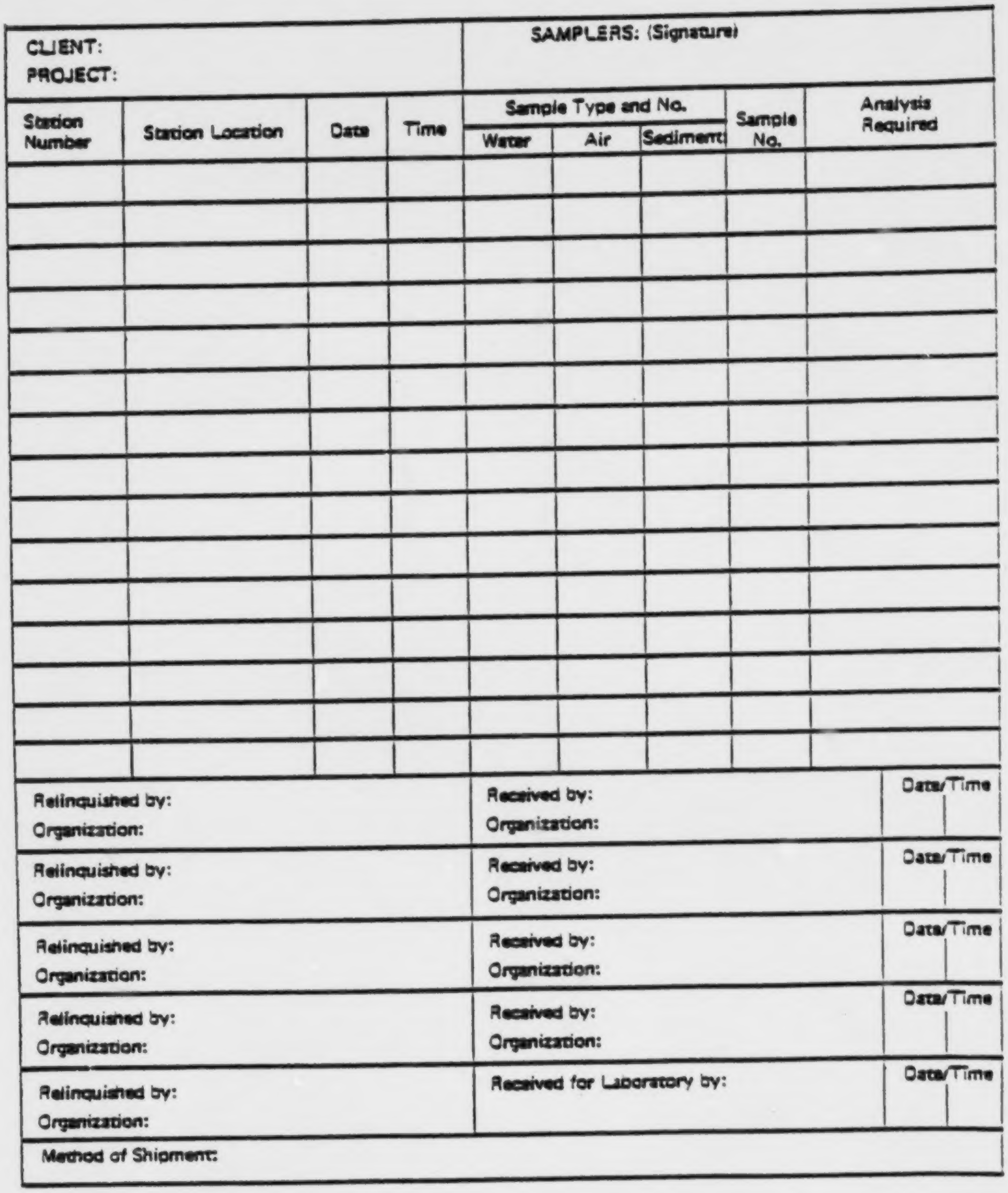
TABLE 4-2. FINAL LIST OF SIC CODE INDUSTRIAL EFFLUENTS SAMPLED FOR EACH PARAMETER AND MATRIX

Exp. no.	Experiment	Matrices M1	 M2	 M3
1-3	Phenols	2911	3312	2211
4	Cyanide	3312	3692	3471
5-8	Mercury	2812	3479	2869
12	Ammonia	2869	2911	3312
13	Fluoride	1475	3334	3339
14	TKN	2873	0259	2869
15	Nitrate plus nitrite	2873	0259	2873
16	Organic carbon	2911	2869	2879
17	Total phosphorus	1475	4911	3312
18	Sulfide	2911	2211	3111

```
ENVIRONMENTAL SCIENCE & ENGINEERING   06/08/81          FIELD LOG SHEET                    PFG   060  LABEL
     PROJECT NUMBER: 81418407          PROJECT NAME: HOLDING TIME STUDY
      SAMPLER NAME:                      GEOGRAPHICAL LOCATION WITHIN PROJECT AREA:401:
     FOL NAME  ED KELLAR                                   STORET MAP #                       727   EPA STATE/COUNTY CODE:
E1H1    104400 D      T   E1H2    104401 D      T   E1H3    104402 D      T   E2H1    104403 D      T   E2H2    104404 D      T
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C

E2H3    104405 D      T   E3H1    104406 D      T   E3H2    104407 D      T   E3H3    104408 D      T   E4H1    104409 D      T
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C

E4H2    104410 D      T   E4H3    104411 D      T   E5H1    104412 D      T   E5H2    104413 D      T   E5H3    104414 D      T
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C

E6H1    104415 D      T   E6H2    104416 D      T   E6H3    104417 D      T   E7H1    104418 D      T   E7H2    104419 D      T
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C

E7H3    104420 D      T   E8H1    104421 D      T   E8H2    104422 D      T   E8H3    104423 D      T   E9H1    104424 D      T
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C

E9H2    104425 D      T   E9H3    104426 D      T   E10H1   104427 D      T   E10H2   104428 D      T   E10H3   104429 D      T
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C

E11H1   104430 D      T   E11H2   104431 D      T   E11H3   104432 D      T   E12H1   104433 D      T   E12H2   104434 D      T
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C

E12H3   104435 D      T   E13H1   104436 D      T   E13H2   104437 D      T   E13H3   104438 D      T   E14H1   104439 D      T
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C

E14H2   104440 D      T   E14H3   104441 D      T   E15H1   104442 D      T   E15H2   104443 D      T   E15H3   104444 D      T
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C

E16H1   104445 D      T   E16H2   104446 D      T   E16H3   104447 D      T   E17H1   104448 D      T   E17H2   104449 D      T
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C

E17H3   104450 D      T   E18H1   104451 D      T   E18H2   104452 D      T   E18H3   104453 D      T           104454 D      T
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C
C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C    C  C  C  C  C  C  C  C
```

Figure 4-3. EXAMPLE LOGSHEET USED FOR FIELD SAMPLES

Figure 4-4. EXAMPLE CHAIN-OF-CUSTODY FORM



CLIENT: **PROJECT:**	**SAMPLERS:** (Signature)

Station Number	Station Location	Date	Time	Sample Type and No.			Sample No.	Analysis Required
				Water	Air	Sediment		

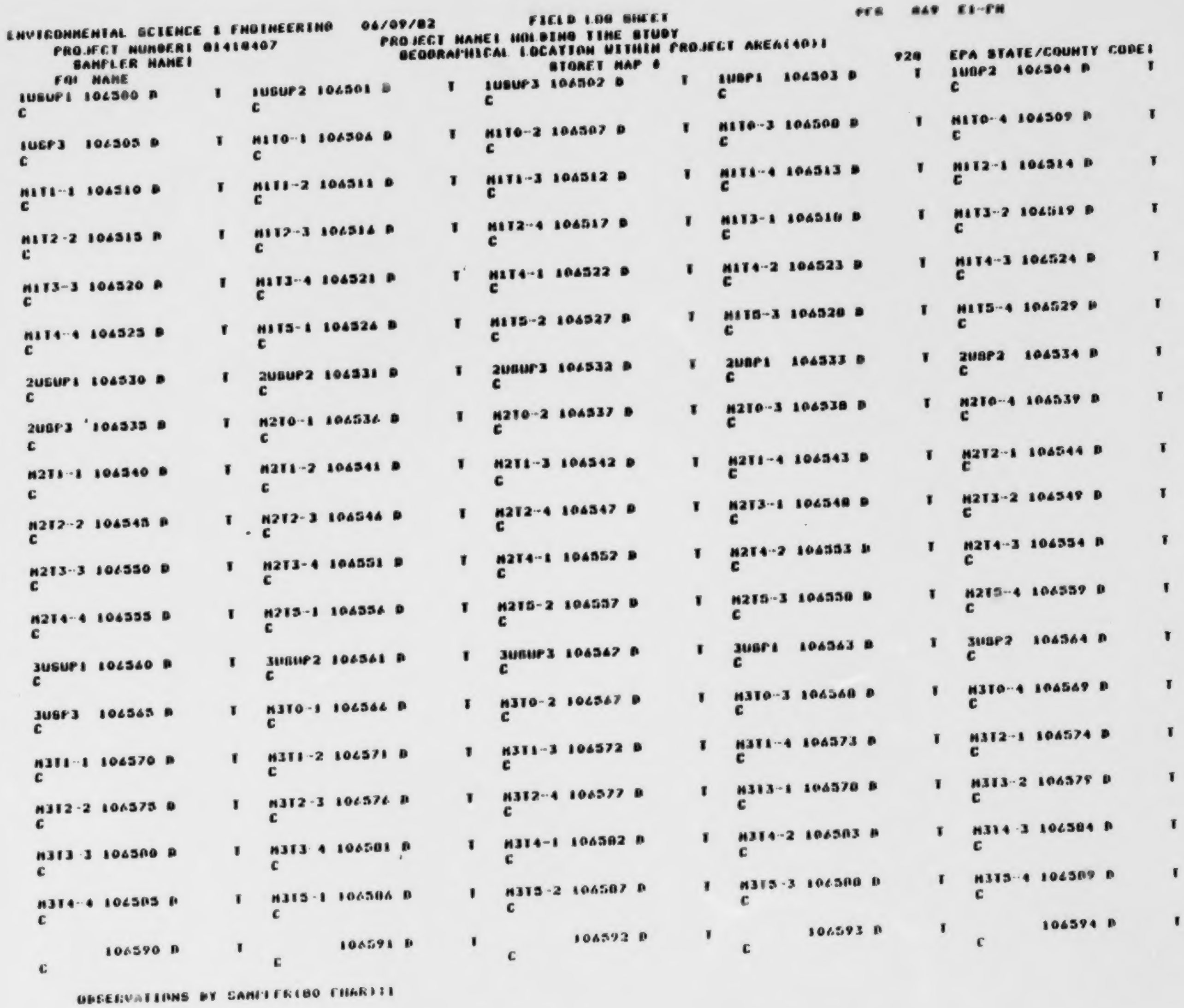
Relinquished by: Organization:	Received by: Organization:	Date/Time
Relinquished by: Organization:	Received by: Organization:	Date/Time
Relinquished by: Organization:	Received by: Organization:	Date/Time
Relinquished by: Organization:	Received by: Organization:	Date/Time
Relinquished by: Organization:	Received for Laboratory by:	Date/Time

Method of Shipment:

```
ESE#81418407   106453  C
HOLDING TIME STUDY
   SAMP#       STAID      FLD.GP.
  106453       E18M3       LABEL
FRACT= C
SAMPLER
SAMP.DATE              HR      M
FGL= ED KELLAR
  106453       E18M3       LABEL
 06/08/81               868-2PFS
```

```
ESE#81418407   106453  C
HOLDING TIME STUDY
   SAMP#       STAID      FLD.GP.
  106453       E18M3       LABEL
FRACT= C
SAMPLER
SAMP.DATE              HR      M
FGL= ED KELLAR
  106453       E18M3       LABEL
 06/08/81               868-2PFS
```

Figure 4-5. EXAMPLE LABELS



```
                                                          FIELD LOG SHEET                          PFS  #49  E1-FM
ENVIRONMENTAL SCIENCE & ENGINEERING   06/09/82      PROJECT NAME: HOLDING TIME STUDY
     PROJECT NUMBER: 81418407                        GEOGRAPHICAL LOCATION WITHIN PROJECT AREA(40):
       SAMPLER NAME:                                                                            928    EPA STATE/COUNTY CODE:
      FG# NAME                                                 STORET MAP #
1USUP1 104500 D      T    1USUP2 104501 D      T    1USUP3 104502 D      T    1UBP1  104503 D      T    1UBP2  104504 D      T
C                         C                         C                         C                         C

1UBP3  104505 D      T    M1T0-1 104506 D      T    M1T0-2 104507 D      T    M1T0-3 104508 D      T    M1T0-4 104509 D      T
C                         C                         C                         C                         C

M1T1-1 104510 D      T    M1T1-2 104511 D      T    M1T1-3 104512 D      T    M1T1-4 104513 D      T    M1T2-1 104514 D      T
C                         C                         C                         C                         C

M1T2-2 104515 D      T    M1T2-3 104516 D      T    M1T2-4 104517 D      T    M1T3-1 104518 D      T    M1T3-2 104519 D      T
C                         C                         C                         C                         C

M1T3-3 104520 D      T    M1T3-4 104521 D      T    M1T4-1 104522 D      T    M1T4-2 104523 D      T    M1T4-3 104524 D      T
C                         C                         C                         C                         C

M1T4-4 104525 D      T    M1T5-1 104526 D      T    M1T5-2 104527 D      T    M1T5-3 104528 D      T    M1T5-4 104529 D      T
C                         C                         C                         C                         C

2USUP1 104530 D      T    2USUP2 104531 D      T    2USUP3 104532 D      T    2UBP1  104533 D      T    2UBP2  104534 D      T
C                         C                         C                         C                         C

2UBP3 '104535 D      T    M2T0-1 104536 D      T    M2T0-2 104537 D      T    M2T0-3 104538 D      T    M2T0-4 104539 D      T
C                         C                         C                         C                         C

M2T1-1 104540 D      T    M2T1-2 104541 D      T    M2T1-3 104542 D      T    M2T1-4 104543 D      T    M2T2-1 104544 D      T
C                         C                         C                         C                         C

M2T2-2 104545 D      T    M2T2-3 104546 D      T    M2T2-4 104547 D      T    M2T3-1 104548 D      T    M2T3-2 104549 D      T
C                         C                         C                         C                         C

M2T3-3 104550 D      T    M2T3-4 104551 D      T    M2T4-1 104552 D      T    M2T4-2 104553 D      T    M2T4-3 104554 D      T
C                         C                         C                         C                         C

M2T4-4 104555 D      T    M2T5-1 104556 D      T    M2T5-2 104557 D      T    M2T5-3 104558 D      T    M2T5-4 104559 D      T
C                         C                         C                         C                         C

3USUP1 104560 D      T    3USUP2 104561 D      T    3USUP3 104562 D      T    3UBP1  104563 D      T    3UBP2  104564 D      T
C                         C                         C                         C                         C

3UBP3  104565 D      T    M3T0-1 104566 D      T    M3T0-2 104567 D      T    M3T0-3 104568 D      T    M3T0-4 104569 D      T
C                         C                         C                         C                         C

M3T1-1 104570 D      T    M3T1-2 104571 D      T    M3T1-3 104572 D      T    M3T1-4 104573 D      T    M3T2-1 104574 D      T
C                         C                         C                         C                         C

M3T2-2 104575 D      T    M3T2-3 104576 D      T    M3T2-4 104577 D      T    M3T3-1 104578 D      T    M3T3-2 104579 D      T
C                         C                         C                         C                         C

M3T3-3 104580 D      T    M3T3 4 104581 D      T    M3T4-1 104582 D      T    M3T4-2 104583 D      T    M3T4-3 104584 D      T
C                         C                         C                         C                         C

M3T4-4 104585 D      T    M3T5-1 104586 D      T    M3T5-2 104587 D      T    M3T5-3 104588 D      T    M3T5-4 104589 D      T
C                         C                         C                         C                         C

       104590 D      T           104591 D      T           104592 D      T           104593 D      T           104594 D      T
C                         C                         C                         C                         C

  OBSERVATIONS BY SAMPLER(80 CHAR):
```

Figure 4-6. EXAMPLE LOGSHEET FOR FILE (FIELD GROUP) SET UP FOR EACH EXPERIMENT

The key for the six-character Station ID codes shown in Figure 4-6 is:
US = Unspiked
UP = Unpreserved
P = Preserved
M1-M3 = Matrix number
T0-T5 = Specific time sequence
Suffix number = Replicate number
Prefix number = Matrix number

After sample mixing and dispensing for a specific sample matrix, a notice of sample lots required for analysis (see Figure 4-7) and the sample lots were sent to the analyst and group leader to ensure proper custody procedures. Sample lots for each analytical run were prepared prior to analysis (see Figure 4-8); six existed for each of the three matrices.

Two additional files were prepared; one was designed to control the additional analyses required for Experiment 10 for nitrate in drinking water (required during the Phase I meeting with EPA in Cincinnati). The other file was prepared to control additional analyses found to be necessary during the course of the experimental work.

4.2.3 Sampling and Transport

With the exception of preservation techniques, sampling and transport were performed according to EPA procedures (1979b). Field preservation included chilling only when required (mercury and fluoride preservation did not require chilling).

Prefield Logistics--

Before a site visit, the Field Team Leader contacted plant personnel at least one day in advance to ensure that plant upsets or sudden shutdowns would not delay field sampling. All field supplies were packaged and shipped with the field team. A checklist of the required and enclosed supplies accompanied the shipment. The Field Team Leader verified proper shipment and safe arrival of shipped items.

A Briefing Package was prepared for each site visited during a field trip to supplement basic instructions provided in a Field Operations Manual. A Field Sampling Plan (see Figure 4-9) and all necessary sample logsheets and labels were provided, after being approved by the Project Manager and QA Supervisor before each field trip.

Container Preparation--

All sampling and shipping containers were prepared according to EPA (1979b), prelabeled, and accompanied by logsheets and chain-of-custody forms. Because changes in unknown matrix parameters due to container preparation could have affected the test parameter of interest, all sample containers (except for the mercury fraction) were prepared to meet the requirements of the most stringent sample fraction (total phosphorus). The container (including caps) preparation for these fractions included nonphosphate laboratory detergent washing, 1:1 hot acid bath with hydrochloric acid (HCl), a deionized water rinse, and air drying. The container and cap preparation for the mercury fraction entailed detergent

TO:

FROM: Karen L. Tuttle, Laboratory
Subproject Manager

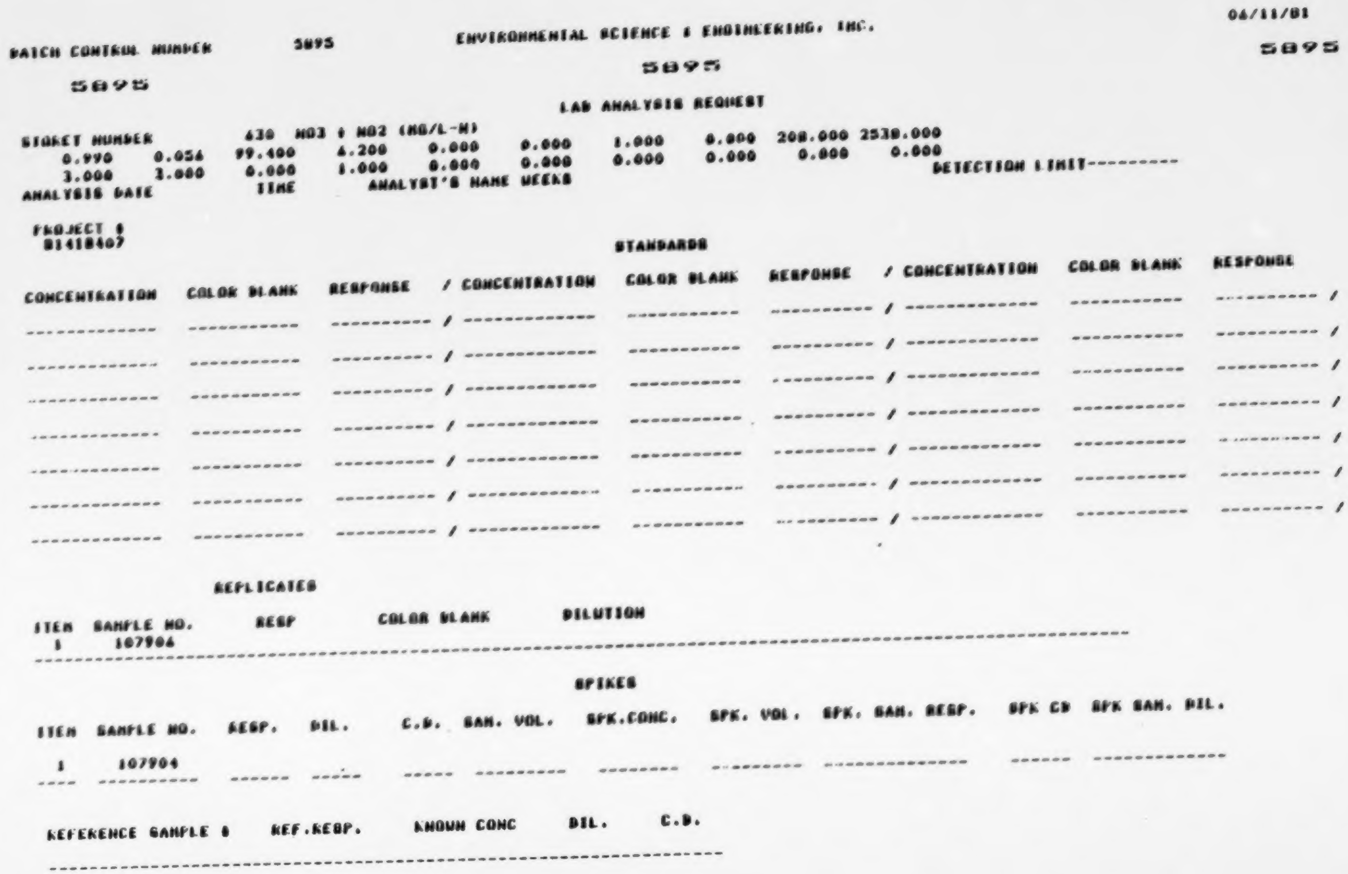
SUBJECT: Batches and Analysis Dates for
Holding Time Samples

The attached batches are ready for analysis. Please analyze them on the dates indicated.

Parameter: Cyanide
Experiment Number: 4
Matrix Number: 1

Batch Number	Analysis Date
6010	8-01-81
6011	8-02-81
6012	8-03-81
6013	8-05-81
6014	8-09-81
6015	8-17-81

Figure 4-7. EXAMPLE MEMORANDUM FOR NOTIFICATION OF SAMPLE LOTS FOR ANALYSIS



```
                                                                                                   06/11/81
BATCH CONTROL NUMBER       5895          ENVIRONMENTAL SCIENCE & ENGINEERING, INC.
                                                                                                   5895
     5895                                         5895
                                           LAB ANALYSIS REQUEST

STORET NUMBER            630  NO3 + NO2 (MG/L-N)
    0.990    0.056   99.400    4.200    0.000    0.000    1.000    0.000  208.000 2530.000
    3.000    3.000    0.000    1.000    0.000    0.000    0.000    0.000    0.000    0.000
ANALYSIS DATE          TIME       ANALYST'S NAME WEEKS                       DETECTION LIMIT---------

 PROJECT #
 81418407                                     STANDARDS
                                                                     / CONCENTRATION  COLOR BLANK  RESPONSE
CONCENTRATION  COLOR BLANK  RESPONSE   / CONCENTRATION  COLOR BLANK  RESPONSE   / -------------  -----------  ---------- /
-------------  -----------  ---------- / -------------  -----------  ---------- / -------------  -----------  ---------- /
-------------  -----------  ---------- / -------------  -----------  ---------- / -------------  -----------  ---------- /
-------------  -----------  ---------- / -------------  -----------  ---------- / -------------  -----------  ---------- /
-------------  -----------  ---------- / -------------  -----------  ---------- / -------------  -----------  ---------- /
-------------  -----------  ---------- / -------------  -----------  ---------- / -------------  -----------  ---------- /
-------------  -----------  ---------- / -------------  -----------  ---------- / -------------  -----------  ---------- /
-------------  -----------  ---------- / -------------

                REPLICATES

ITEM  SAMPLE NO.      RESP        COLOR BLANK       DILUTION
  1    107904
------------------------------------------------------------------------------------------

                                          SPIKES

ITEM  SAMPLE NO.  RESP.  DIL.    C.B.  SAM. VOL.  SPK.CONC.  SPK. VOL.  SPK. SAM. RESP.  SPK CB  SPK SAM. DIL.

  1    107904
----  ----------  ------ -----   ----- ---------  ---------  ---------  ---------------  ------  ------------

 REFERENCE SAMPLE #   REF.RESP.   KNOWN CONC    DIL.    C.B.
 ---------------------------------------------------------------
```

Figure 4-8. EXAMPLE SAMPLE LOT (BATCH) FORM FOR REPORTING ANALYSIS AND QC INFORMATION (Page 1 of 2)

SAMPLE DATA

ITEM	SAMPLE NO.	RESP.	DIL.	C.B.
1	107900			
2	107901			
3	107902			
4	107903			
5	107904			
6	107905			
7	107906			
8	107907			
9	107908			
10	107909			

Figure 4-8. EXAMPLE SAMPLE LOT (BATCH) FORM FOR REPORTING ANALYSIS AND QC INFORMATION (Page 2 of 2)

FIELD SAMPLING PLAN
HOLDING TIME PROJECT
Trip ____
Sample Matrices to be Collected: ____________________

Date: ____________________

Project Manager: Hugh Prentice

Subproject Manager: Ed Kellar

Field Team Leader: ____________________

Field Team: ____________________

Itinerary:
  Date Departing: ____________________
  Accommodations: ____________________
  Phone: ____________________

Sample Matrix	Date to be Shipped	How Shipped	Time Samples Need to be Shipped	Time of Arrival at Gville.	No. of Containers	Time of Confirmation Call

cc: Karen Tuttle
    Hugh Prentice
    Project File

Figure 4-9. EXAMPLE FIELD SAMPLING PLAN

washing, rinsing with 1:1 nitric acid (NHO_3), then 1:1 HCl, and, finally, deionized water.

All field equipment that could have come in contact with a wastestream was prepared using the same technique. In the field, equipment was dedicated to a specified sample site to eliminate any cross-contamination of samples.

Sample Collection--

Except for one site, all sample points were NPDES Sample Points of Record. Sulfide in a leather tanning and finishing effluent (SIC Code 3111) was discharged into a municipal wastewater treatment and did not require compliance monitoring. Plant personnel were familiar with the sample point and its performance history.

Only grab samples were taken due to the extremely large sample volume required per sample point (see Table 4-3) and because they were satisfactory for these experiments. This necessitated selection of well-mixed and equalized sampling points. Discussions with plant personnel prior to the field trip ensured that sample collection was scheduled during normal operating conditions. Grab sampling ensured the most rapid collection for timely shipment of samples for analysis.

Each sample was collected as a series of 2.5-gallon glass or 5-gallon plastic fractions (see Table 4-3) taken from mid-depth, mid-stream in the NPDES outfall. Immediately after the last fraction was taken, the samples were packed for shipment.

Field Documentation--

All samples collected in the field were labeled and logged by the Field Team Leader using the labels, sample logsheets, and the chain-of-custody forms prepared by the computerized ESE Data Management System before sample collection. Field notebooks and copies of all logsheets were submitted for Subproject and Project Manager review at the conclusion of each field trip. The Field Team Leader was responsible for reviewing all field notes for completeness and signing them before leaving the field; the project QA Supervisor audited all field notes.

Preservation, Packing, and Shipment of Samples--

Field preservation was limited to chilling of samples so that the effect or absence of effect of the preservative could be measured. Insulated metal coolers with internal shockproofing were packed with double-bagged ice to maintain each aliquot at 4 degrees Celsius (°C) for at least 24 hours. The Field Team Leader and Subproject Manager selected the most expeditious means of transportation before arrival at the sample site. In all cases, the prearranged shipping method was selected to guarantee safe arrival and preparation of the matrix to provide for analysis of the spiked analyte within 48 hours after completion of sampling. Where necessary, sampling times were coordinated with shippers' schedules to avoid shipping delays. For field locations within driving distance of the laboratory, ESE delivered all samples. An ESE courier was on call to immediately obtain

TABLE 4-3. FIELD SAMPLE VOLUMES REQUIRED FOR EACH EXPERIMENT

Parameter	Experiment number	Minimum volume to be sampled (gal)	Type container*	Minimum number of 2.5-gal glass or 5-gal plastic containers
Phenols	1	27	P	6
	2	27	G	11
	3	27	G	11
Cyanide	4	27	P	6
Mercury	5	11	P	3
	6	11	G	5
	7	11	G	5
	8	11	P	3
Nitrate	9	7	P	2
	10	7	P	2
Fluoride	11	7	P	2
Ammonia	12	27	P	6
Fluoride	13	18	P	4
TKN	14	27	P	6
Nitrate plus nitrite	15	7	P	2
Organic carbon	16	7	P	2
Total phosphorus	17	7	P	2
Sulfide	18	10	P	6

*P = plastic, G = glass.

samples from the shipping agency. In all cases, shipping and packaging procedures complied with EPA and United States Department of Transportation (DOT) procedures.

4.2.4 Sample Receiving, Preparation for Analysis, and Storage

As stated in Section 4.2.3, the Laboratory Operations Subproject Manager was notified of sample shipment method and schedule. Upon sample arrival at the laboratory, completed logsheets and chain-of-custody forms were signed and submitted to the Laboratory Data Assistant for entry into the computerized data management system. The container preparation for mixing and the dispersing apparatus was the same as described in Section 4.2.3 for field sampling. Table 4-4 is the checklist of containers and glassware used for mixing and dispensing. Table 4-5 presents the sample volume required for the total sample and sample aliquots.

Homogenizing Field Samples--

Sample fractions received from the field were composited in a calibrated glass or plastic vat large enough to contain the entire sample (see Table 4-4) and mixed until the sample was considered homogeneous. Figure 4-10 depicts the mixing device used. A cover and nitrogen gas were required for sulfide sample matrices to prevent sulfide oxidation to sulfate.

The 100-milliliter (mL) aliquots were withdrawn from the mixing vat at selected time intervals before and after dispensing samples for time-sequenced analyses. These aliquots were used to determine the pH, turbidity, and conductivity to evaluate the homogeneity of the mixed sample. The sample, which was considered homogeneous when three successive pH, turbidity, and conductivity measurements differed by less than 10 percent, was disposed of after testing. Data were documented on the form shown in Figure 4-11.

Tables 4-6 and 4-7 summarize the preservatives and spiking solutions used for each time-sequenced experiment. The volumes remaining in the qualitatively calibrated mixing vats were used to determine the appropriate spike addition. Due to the 48-hour time limit from sample collection to time-zero analysis, it was not practical to analyze unspiked samples to determine the desired spike level. Volumes needed for each spiking solution to achieve the desired final concentration were determined from historical data of the ambient discharge concentration at each matrix sample site.

Choosing Aliquots for Analysis--

After the homogenized spiked and preserved matrix had been dispensed and labeled, samples were chosen randomly for the time-sequenced analysis and the chosen aliquots relabeled immediately. Aliquots chosen for time-zero analysis were fixed instead of randomly chosen (Aliquots 1, 50, and 100). These samples were used to check for homogeneity of the analyte among the dispensed samples.

TABLE 4-4. CHECKLIST OF CONTAINERS AND GLASSWARE NEEDED

Parameter	Experiment number	Container for spiking	Container for mixing	Container for storage*
Phenols	1	Volumetric flask	30-gal plastic vat	1-l Cubitainer®
	2	Volumetric flask	13-gal glass vat	1-quart Mason jar
	3	Volumetric flask	13-gal glass vat	1-quart Mason jar
Cyanide	4	Volumetric flask	30-gal plastic vat	1-l Cubitainer®
Mercury	5	Volumetric flask	13-gal glass vat	1-l Cubitainer®
	6	Volumetric flask	13-gal glass vat	1-l Cubitainer®
	7	Volumetric flask	13-gal glass vat	1-l Cubitainer®
	8	Volumetric flask	13-gal plastic vat	1-l Cubitainer®
Nitrate	9	Volumetric flask	10-gal plastic vat	60-mL snaptop vial
	10	Volumetric flask	10-gal plastic vat	60-mL snaptop vial
Fluoride	11	Volumetric flask	10-gal plastic vat	1-l Cubitainer®
Ammonia	12	Volumetric flask	30-gal plastic vat	1-l Cubitainer®
Fluoride	13	Volumetric flask	30-gal plastic vat	1-l Cubitainer®
TKN	14	Volumetric flask	30-gal plastic vat	1-l Cubitainer®
Nitrate plus nitrite	15	Volumetric flask	10-gal plastic vat	60-mL snaptop vials
Organic carbon	16	Volumetric flask	10-gal plastic vat	60-mL snaptop vials
Total phosphorus	17	Volumetric flask	10-gal plastic vat	60-mL snaptop vials
Sulfide	18	Volumetric flask	7-gal plastic vat	60-mL snaptop vials

* One hundred containers were needed for each of the three matrices for each experiment except Experiments 2 and 3; those required 50 to avoid the use of a large unsafe glass vat.

TABLE 4-5. SAMPLE VOLUME REQUIRED FOR EACH EXPERIMENT

Parameter	Experiment number	Number of aliquots	Volume per aliquot (mL)	Minimum sample volume required (L)	Total volume to be sampled (gal)	Type container*	Size container (gal)
Phenols	1	106	750	79.5	27	P	30
	2	56†	750	79.5	11	G	13
	3	56†	750	79.5	11	G	13
Cyanide	4	106	750	79.5	27	P	30
Mercury	5	106	250	26.5	11	G	13
	6	106	250	26.5	11	G	13
	7	106	250	26.5	11	G	13
	8	106	250	26.5	11	P	13
Nitrate	9	106	60	10.6	7	P	10
	10	106	60	10.6	7	P	10
Fluoride	11	106	200	21.2	7	P	13
Ammonia	12	106	750	79.5	27	P	30
Fluoride	13	106	500	53.0	18	P	30
TKN	14	106	750	79.5	27	P	30
Nitrate plus nitrite	15	106	60	10.6	7	P	10
Organic carbon	16	106	60	10.6	7	P	10
Total phosphorus	17	106	60	10.6	7	P	10
Sulfide	18	106	60	79.5	27	P	10

*P = plastic, G = glass.

†Smaller volume needed because a 30-gallon glass mixing vat was unsafe.

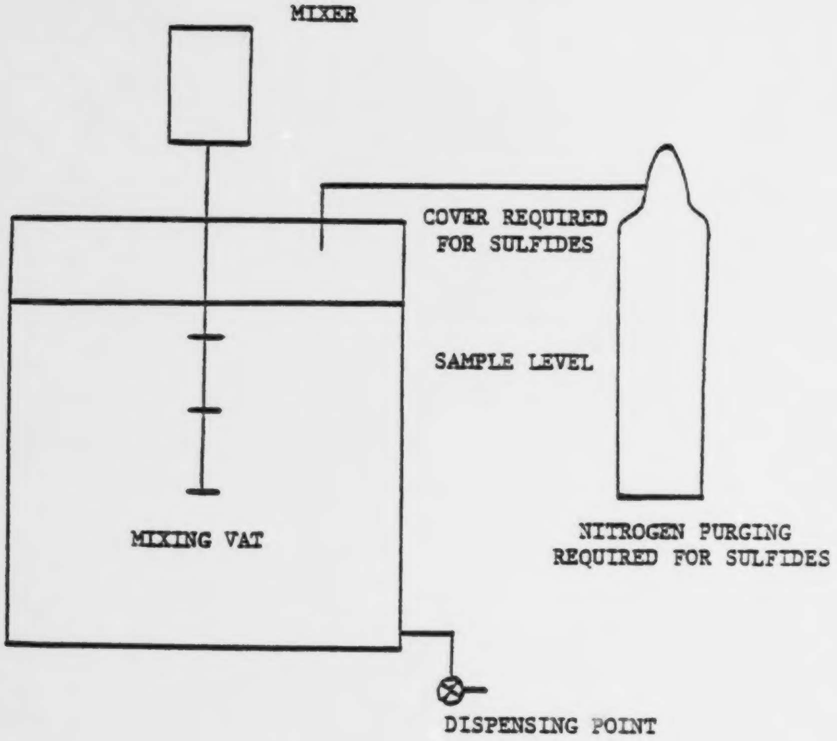

MIXER
COVER REQUIRED FOR SULFIDES
SAMPLE LEVEL
MIXING VAT
NITROGEN PURGING REQUIRED FOR SULFIDES
DISPENSING POINT

Figure 4-10. MIXING DEVICE FOR HOMOGENIZED SAMPLE

Homogeneity Documentation Form

Experiment No. __________
Parameter __________
Matrix __________
Time Mixing Initiated __________

Time After Mixing Starts	Temperature	Conductivity	Turbidity	pH

Figure 4-11. EXAMPLE HOMOGENEITY DOCUMENTATION FORM

TABLE 4-6. PRESERVATION TECHNIQUES FOR EACH EXPERIMENT

Parameter	Experiment number	Preservation technique
Phenols	1	Cool 4°C, H_2SO_4 pH<2
	2	Cool 4°C, 1.0 g/L $CuSO_4$, H_3PO_4 pH<4
	3	Cool 4°C, H_2SO_4 pH<2
Cyanide	4	Cool 4°C, NaOH pH>12
Mercury	5	HNO_3 pH<2, 0.05% $K_2Cr_2O_7$
	6	HNO_3 pH<2, 0.05% $K_2Cr_2O_7$
	7	HNO_3 pH<2
	8	HNO_3 pH<2
Nitrate	9	H_2SO_4 pH<2
	10	Cool 4°C
Fluoride	11	None
Ammonia	12	Cool 4°C, H_2SO_4 pH<2
Fluoride	13	None
TKN	14	Cool 4°C, H_2SO_4 pH<2
Nitrate plus nitrite	15	Cool 4°C, H_2SO_4 pH<2
Organic carbon	16	Cool 4°C, H_2SO_4 pH<2
Total phosphorus	17	Cool 4°C, H_2SO_4 pH<2
Sulfide	18	Cool 4°C, 2 mL/L of 2N zinc acetate

TABLE 4-7. CONSTITUTION OF MATRIX SPIKES

Parameter	Experiment number	Constitution of spike	Concentration of spike solution (mg/L)*
Phenols	1, 2, and 3	Phenol	1,000
Cyanide	4	KCN + KOH	1,000
Mercury	5, 6, 7, and 8	$HgCl_2$ (AAS standard solution for element mercury)	1,000
Nitrate	9 and 10	KNO_3	1,000
Fluoride	11	NaF	100
Ammonia	12	NH_4Cl	1,000
Fluoride	13	NaF	100
TKN	14	NH_4Cl	1,000
Nitrate plus nitrite	15	KNO_3	1,000
Organic carbon	16	$KHC_8H_4O_4$	1,000
Total phosphorus	17	KH_2PO_4	100
Sulfide	18	$Na_2S.9H_2O$	50

* mg/L = milligrams per liter.

4.2.5 Sample Analysis

Only EPA-approved analytical procedures (EPA, 1979b) were used for this project. Table 4-8 summarizes the analytical methods and references used for each experiment. Use of the automated-phenate method for analysis of ammonia in the distillate of the digested TKN samples was not specifically defined in the Federal Register (EPA, 1979b); however, approval was received by EPA (prior to implementation).

The analyst performed the following quality control (QC) checks to meet the documented QC criteria:

1. The analyst prepared the required calibration curve (five standards plus a blank) and calculated the slope.
2. Instrument calibration was completed by ensuring that the slope and a known reference were within control limits.
3. The actual samples, blind control samples, spiked samples, and duplicate samples were then analyzed.
4. After every 10 samples, and/or at the end of the run, the mid-range calibration standard was analyzed to document instrument drift and determine whether correction factors were necessary.

After these QC checks, the data sheets for sample lots (Figure 4-8) were submitted for entry into the computer data management system for calculation of raw data, reference samples, and blind control samples (see Section 4.2.3).

4.2.6 Quality Assurance

A project-specific QA plan was developed to ensure that project performance met the required objectives outlined in the contract. Under the direction of the project QA Supervisor, audits, controls, and validation procedures were implemented to assess data precision, accuracy, and completeness.

Assessment Procedures for Precision, Bias (Accuracy), and Completeness--

Precision, bias (accuracy), and completeness were assessed using the following procedures. Precision was assessed three ways. The instrument drift for each measurement system was checked by running one standard after every 10 samples and/or at the end of the run. Analytical reproducibility was assessed by analyzing 10 percent replicate samples for each sample lot. Experimental variability was determined for each day of analysis by analyzing triplicate aliquots; these data were used during the statistical evaluation. Data bias (accuracy) was assessed for each measurement system and each sample lot on three different samples: a known reference sample, a blind reference sample, and a spiked sample aliquot. Data completeness was assessed on a sample lot basis. The study was designed with equal cell frequencies; therefore, data completeness could be monitored directly.

Before accepting data in the computer data files for each field group (see Section 4.2.2), the following QC criteria were met:

1. At least five standards were used for standard curve;
2. Correlation coefficient for curve was greater than criteria;
3. Coefficient of variation for replicates was within criteria;

TABLE 4-8. ANALYTICAL METHODS

Parameter	Method	Reference EPA method number*
Ammonia (as N)	Manual distillation (at pH 9.5) followed by automated phenate	350.1
Cyanide, total	Distillation followed by pyridine-barbituric colorimetric analysis	335.2
Fluoride	Distillation followed by ion electrode no distillation required for drinking water samples	340.2
TKN (as N)	Digestion and distillation followed by automated phenate	351.1
Mercury, total	Manual cold vapor flameless AA	245.1
Nitrate (as N)	Automated cadmium reduction	353.2
Nitrate plus nitrite (as N)	Automated cadmium reduction, automated colorimetric analysis	353.2
Organic carbon, total	Combustion, infrared method	415.1
Phenols	Distillation followed by 4-amino antipyrine colorimetric analysis	420.1
Phosphorus, total (as P)	Persulfate digestion, manual ascorbic, and reduction	365.2
Sulfide (as S)	Methylene blue colorimetric analysis	376.2

* EPA, 1979a.

4. Percent recovery for spikes was within criteria;
5. Y-intercept was less than two times detection limit;
6. Samples or dilutions were within range of standards;
7. At least 10 percent of the samples were replicates; and
8. At least 5 percent of the samples were spiked.

Systems and Performance Audits--

When each operating system was working properly, performance audits were conducted. The performance audit protocol included:

1. Site Selection--The project QA Supervisor monitored the site selection process to ensure that representative sites were chosen.
2. Field Operations--One field audit of the field operator's collection and shipping procedures was performed by the project QA Supervisor. In addition, the project QA Supervisor checked:
    a. Field notebooks, logsheets, and tracking forms, and reported any inconsistencies and/or omissions;
    b. Field sampling plans; and
    c. Sample site briefing package.
3. Laboratory Operations--The project QA Supervisor monitored:
    a. Sample log-in;
    b. Homogenizing of the samples;
    c. Dispensing and labeling of samples for analysis;
    d. Updating of QC charts of the blind control samples; and
    e. Final approval of each sample lot.

    In addition, the project QA Supervisor monitored all experiments to assure complete adherence to the analytical methods.
4. Statistical Evaluation--The project QA Supervisor monitored the output of statistical programs to ensure integrity of results.
5. Final Report--The project QA Supervisor reviewed the final report.

Data Reduction, Reporting and Validation--

Upon completion of analysis and instrument QC checks, the sample lot form was completed by the analyst, approved by the Technical Supervisor, and sent to the Laboratory Data Assistant. The Laboratory Data Assistant entered the information into the computer, for the following calculations:

1. Quadratic regression line for standard curve,
2. Coefficient of variation for replicates,
3. Spiked recoveries,
4. Reference sample concentrations, and
5. Sample concentrations.

The quadratic regression line for the standard curve, used to calculate results for analyses requiring a calibration curve, resulted in the following equation:

$$\text{Concentration} = \text{Intercept} + \text{D}(\text{Response}) + \text{D2}(\text{Response})^2 \tag{1}$$

where:

$$\text{Intercept} = \frac{\text{CO}\,[(\text{AB2})(\text{AB4}) - (\text{AB3})^2] + \text{COAB}\,[(\text{AB2})(\text{AB3}) - (\text{AB})(\text{AB4})] + \text{AB2CO}\,[(\text{AB})(\text{AB3}) - (\text{AB2})^2]}{\text{Denominator}}$$

$$D = \frac{N\,[(AB4)(COAB) - (AB3)(AB2CO)] - (AB)(AB4)(CO) + AB2\,[(AB)(AB2CO) + (AB3)(CO) - (AB2)\,(COAB)]}{\text{Denominator}}$$

$$D2 = \frac{N\,[(AB2)(AB2CO) - (AB3)(COAB)] + AB\,[(AB2)(COAB) + (AB3)(CO) - (AB)(AB2CO)] - (AB2)^2(CO)}{\text{Denominator}}$$

$$\text{Denominator} = N[(AB2)(AB4) - (AB3)^2] + (2AB)(AB2)(AB3) - (AB2)^3 - (AB)^2(AB4)$$

$$\text{Correlation Coefficient} = \frac{M[(N)(COAB) - (AB)(CO)] + M2[(N)(AB2CO) - (AB2)(CO)]}{N(CO2) - (CO)^2}$$

D = Coefficient,
D2 = Coefficient,
AB = Sum(absorbances),
AB2 = Sum$(\text{absorbances})^2$,
AB3 = Sum$(\text{absorbances})^3$,
AB4 = Sum$(\text{absorbances})^4$,
CO = Sum(concentration),
CO2 = Sum$(\text{concentration})^2$,
COAB = Sum(concentration x absorbance),
AB2CO = Sum[$(\text{absorbance})^2$ x concentration], and
N = Number of standards.

The coefficient of variation for replicates was calculated as follows:

$$\text{Coefficient of Variation} = \left|\frac{(\text{Rep 1 Conc} - \text{Rep 2 Conc})}{(\text{Rep 1 Conc} + \text{Rep 2 Conc})}\right| \qquad (2)$$

Spike recoveries were calculated as follows:

$$\text{Actual Recovery} = 100 \times \frac{(\text{Spike Sample Conc.})(\text{Sample+Spike Vol.}) - (\text{Sample Vol.})(\text{Sample Conc.})}{(\text{Spike Conc.})(\text{Spike Volume})} \qquad (3)$$

The following is an example of how this is calculated:

$$100 \times \frac{(20)(11)-(10)(10)}{(1)(100)} = 120 \text{ percent}$$

for:

1 mL of spike with concentration 100 parts per billion (ppb)
10 mL of sample with concentration 10 ppb
spiked sample concentration of 20 ppb

At the completion of each analytical sample lot, the Sample Lot Form (see Figure 4-6) was completed and submitted to the Technical Supervisor (Task Manager). He in turn reviewed all data and documentation, either approved the data or called for a review, and then routed the information to the Laboratory Data Assistant for entry into the computer system. Subsequently, the data were routed to the Project Manager and the project QA Supervisor for review and final approval.

At the end of all experiments, computer data files were generated and used as data input files for the computer statistics program. Each of these files was validated by both the Project Manager and the project QA Supervisor to ensure accurate data were used for the statistical analyses. The statistical computer model was validated using hand calculations and by the development of a second computer program for comparison of results. Appendix A provides a complete documentation of the computer programs.

4.2.7 Protocol for Statistical Tests and Logic

The purpose of this study was to experimentally determine MHT's for various preservation and storage procedures of samples collected for analysis of certain constituents. A statistical protocol (Appendix) was developed to evaluate the suitability of a zero or first-order kinetic model to calculate a CT and its 90 percent LCL (or MHT). Only the zero-order model (concentration versus time) and the first-order model (natural log of concentration versus time) were evaluated since loss or gain of the constituent was expected to follow zero- or first-order kinetics. Figure 4-12 graphically summarizes the protocol for evaluating the model and calculating the statistics on the data set to obtain an MHT.

A computer program was developed to perform the three steps of the protocol using the Statistical Analysis System (SAS) computer package (Appendix). Individual computer programs were written in FORTRAN to verify the SAS computer program. The three steps involved choice of model, evaluation of linear fit, and calculation of the CT and the 90 percent LCL or MHT. If the three steps were satisfactorily completed, the experimentally determined MHT was specified as either the 90 percent LCL of the calculated CT or the maximum time of the experiment.

CHOICE OF MODEL

For each model, two-way analyses of variance (ANOVA) were performed for an experimental data set of three sample matrices. These ANOVA's yielded residuals for each matrix-time combination which were then pooled. Although these residuals do not contain as much information as the residuals from the actual regression analysis, when examined in conjunction with the lack of linear fit, they were judged to provide adequate information for the choice of a model. Essentially, each residual is the difference between each observation and its corresponding cell mean for the triplicate analyses on any one day. An analysis of these residuals was performed for both the zero-order and first-order models to determine which model better satisfied the assumption of normality and homogeneity of the residuals. The basic interpretive steps for examining the two-way ANOVA results to determine which model provided the best fit of the data were:

1. Qualitative examination of histograms to judge which model produced residuals which appear normally distributed;
2. Comparison of significance levels for test of normality; and
3. Qualitative examination of boxplots of the residuals for each matrix to judge which model produced residuals with more homogeneous variances and lack of statistical outliers.

The observations at Day 32 strongly influence the best-fit line. For some parameters, observations were made beyond Day 32 to verify the observation on Day 32.

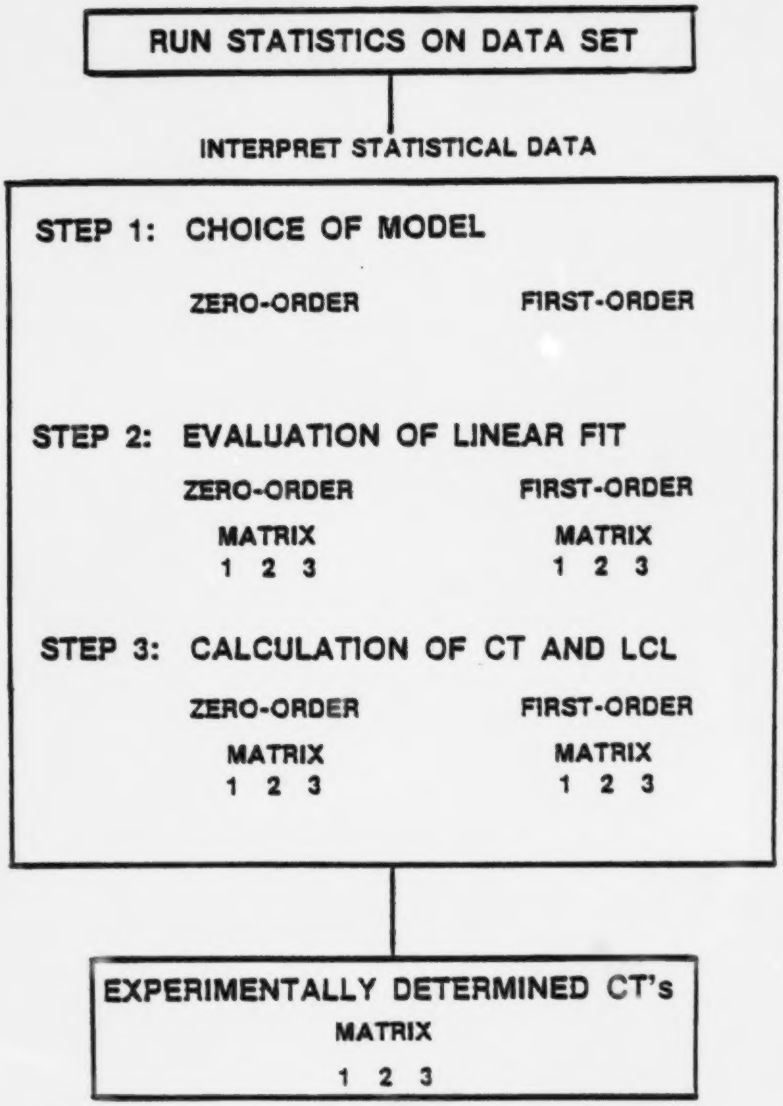

RUN STATISTICS ON DATA SET
INTERPRET STATISTICAL DATA
STEP 1: CHOICE OF MODEL
ZERO-ORDER
FIRST-ORDER
STEP 2: EVALUATION OF LINEAR FIT
ZERO-ORDER
FIRST-ORDER
MATRIX
1 2 3
MATRIX
1 2 3
STEP 3: CALCULATION OF CT AND LCL
ZERO-ORDER
FIRST-ORDER
MATRIX
1 2 3
MATRIX
1 2 3
EXPERIMENTALLY DETERMINED CT's
MATRIX
1 2 3

Figure 4-12 FLOW CHART SUMMARIZING STATISTICAL PROTOCOL

EVALUATION OF LINEAR FIT

Six lack-of-fit ANOVA's were performed for each experimental data set, one for each matrix-order combination. The purpose of these ANOVA's was to provide information indicating whether data trends (concentration or log concentration versus time of analyses) were best explained by a straight line, thereby justifying the use of the model for calculating CT and its LCL.

Results for two types of statistical tests were provided by each ANOVA. The null hypothesis for the first test ("linear" term) stated that there was zero slope for the best-fit regression line. The null hypothesis for the second test ("lack-fit" term) stated that the trend of the data was best explained by a straight line. The alternative hypothesis stated that a higher-order model is needed to model concentration changes with time. Low probabilities (below 0.1 significance level) were evidence to reject the null hypothesis.

The lack-of-fit test compares the error around the straight line to the within-day variability. A significant lack of fit means one of two things or a combination of both occurs.

1. The day-to-day analytical variability was greater than within-day variability; this was usually the case in these experiments.
2. A higher-order model is needed to identify the relationship between concentration changes with time; in these cases, the Duncan's multiple range test comparisons can at least locate the range of days in which the MHT occurs.

In most cases, a lack of linear fit was indicated. A significant lack of fit calls into question the assumptions necessary for testing the hypothesis that the slope is equal to zero and for setting up confidence intervals.

Therefore, when a lack of linear fit was indicated, the data were checked for systematic deviations from linearity (for example, an exponential decrease in concentration). If the lack of linear fit appeared due to random day-to-day analytical variability, the percent standard deviation (CV) derived from the unexplained variability (sum of squares of the lack-of-fit error) was compared to expected (published) analytical variability prior to assuming that the calculated CT's and LCL's were valid. Analytical variability estimates, however, are not widely published and vary from lab to lab and from procedure to procedure. This made the criterion for comparing lack-of-fit variability to expected analytic variability difficult to define precisely. As a general rule, as long as the CV of the lack of linear fit term was lower than 20 percent, and seemed in line with the available published variability, a lack of linear fit was not considered significant. The CV of the unexplained variability (representing day-to-day analytical variability) was calculated as follows:

Zero-order model:

$$\text{Lack-fit CV} = \frac{100(\text{SS/DF})^{1/2}}{\text{c}} \qquad (4)$$

where:

SS = Sum of squares of error for the lack-fit term,
DF = Degrees of freedom for the lack-fit term, and
$\bar{c}$ = Overall mean concentration.

First-order model:

$$\text{Lack-fit CV} = 100(SS/DF)^{1/2} \tag{5}$$

CALCULATION OF CT AND LCL (MHT)

Two regression analyses were performed, one for each order, pooling all sources of error. The first step towards specifying the experimentally determined MHT was to evaluate the slope test statistic. The null hypothesis for the slope test stated that there was zero slope for the best-fit regression line. The alternative hypothesis stated that there was a slope. Low probabilities (below 0.1 significance level) were evidence to reject the null hypothesis. High probabilities (greater than 0.1 significance level) suggested that the experimentally determined MHT be taken as the maximum time of the experiment if the model seemed appropriate, that is, if the unexplained variability of the lack-fit term was within expected analytical error (generally less than 20 percent).

For cases with significant slopes (low probabilities), another statistical test was performed. This test determined whether a 10 percent change in the intercept (concentration at time zero) of the best-fit regression line represented a statistically significant difference. The test compared the lower 90 percent confidence limit of the intercept (A-1.75 x standard error of the intercept) with 90 percent of the intercept (0.9A). When 90 percent of the intercept was smaller than the lower 90 percent confidence limit, a 10 percent change was significant. When 90 percent of the intercept was larger, the significant percent concentration change was taken to be the lower 90 percent confidence limit of the intercept, calculated as follows for the zero-order case:

$$\text{Relative } X = 100 \; \frac{1.75 \times SE}{A} \tag{6}$$

where:

X = The percent change significant at the 90 percent confidence level,
SE = Standard error of the intercept,
1.75 = The t statistic corresponding to the 90 percent confidence level and 16 degrees of freedom, and
A = Intercept of best-fit regression line.

For the first-order case, the significant percent concentration change was calculated by the approximation:

$$X = 100 \times 1.75 \times SE \tag{7}$$

Following the choice of model, evaluation of linear fit, test of slope, and test of significant difference for a 10 percent or greater concentration change, the calculated CT and LCL were determined as follows:

Zero-order model:

$$CT = \pm 0.1 \; \frac{A}{K} \tag{8}$$

$$LCL = CT - 1.34(VAR\_CT)^{1/2} \tag{9}$$

where:

K = Slope of best-fit regression line in units of concentration change per day,

VAR_CT = Variance of CT in units of days squared = the approximation:

$$\frac{(0.01 \times VAR\_A+0.2 \times CT \times COV\_AK+CT^2 \times VAR\_K)}{K^2}$$

VAR_A = Variance of intercept,

COV_AK = Covariance between intercept and slope,

VAR_K = Variance of slope, and

1.34 = The one-tailed t value corresponding to 90 percent confidence level and 16 degrees of freedom.

First-order model:

$$CT = \frac{Ln(0.9)}{K} \text{ or } \frac{Ln(1.1)}{K} \tag{10}$$

$$LCL = CT-1.34(VAR\_CT)^{1/2} \tag{11}$$

where:

Ln = Natural logarithm,

K = Slope of best-fit line in units of inverse days, and

VAR_CT = Variance of CT in units of days squared =

$$\frac{(CT^2 \times VAR\_K)}{K^2}, \text{ and}$$

VAR_K = Variance of slope.

When the percent concentration difference at the 90 percent confidence level was significant at a level greater than 10 percent, but less than 15 percent, that was used in the calculated CT equations for both zero- and first-order cases. The experimentally determined MHT was specified as either the 90 percent LCL of the calculated MHT or the maximum time of the experiment.

SECTION 5.0

RESULTS AND DISCUSSION

The results of the 18 MHT experiments performed during this study are presented and discussed. Ten parameters (phenol, cyanide, mercury, nitrate/nitrite, fluoride, ammonia, TKN, organic carbon, total phosphorus, and sulfide) were evaluated. Experiments for nitrate and fluoride were conducted using both wastewater discharge from three different NPDES sources and treated drinking water from three different sources, while experiments for the other eight parameters used only wastewater discharge. Each experiment included time series tests for concentration changes of the specified parameter. Multiple experiments for phenols, mercury, and nitrate were conducted to determine which preservation technique provided the longest experimentally determined MHT's.

A summary of the results is presented in Table 5-1. The experimentally determined MHT's resulting from this study were longer than the proposed MHT values published in the Federal Register (EPA, 1979c) and recommended by EPA (1979a) for the parameters in wastewater listed below.

Phenols
Ammonia
Fluoride
TKN

Nitrate plus nitrite
Total organic carbon
Total phosphorus
Sulfide

Mercury and cyanide experiment results, however, did not support proposed and recommended MHT's for all sample matrices; these parameters are discussed in further detail.

The experimentally determined MHT's for fluoride and nitrate in drinking water were longer than the EPA (1978)-required values and are summarized in Table 5-1. Table 5-2 summarizes the approximate parameter concentrations studied for each sample matrix.

In almost every case during the statistical analysis of the experimental data sets, the lack-of-fit term had low probabilities requiring an evaluation of the source of the unexplained variability. Table 5-3 presents the CV's for each experiment and matrix. Approximately 57 percent of the experiment/matrix combinations showed CV's lower than 10 percent while 91 percent had CV's lower than 19 percent. CV's greater than 19 percent were observed only in the phenols and mercury experiments.

TABLE 5-1. SUMMARY OF EXPERIMENTALLY DETERMINED MHT's

Experiment number	Parameter	Preservation technique	Proposed MHT's (days)	Experimentally determined MHT in matrices*		
				M1	M2	M3
Wastewater parameters						
1	Phenols	Cool 4°C H_2SO_4 $pH<2$	28	32	32	32
2	Phenols	Cool 4°C 1 g/L $CuSO_4$ H_3PO_4 $pH<4$	1†	32	5(8)	3(4.2)
3	Phenols	Cool 4°C H_2SO_4 $pH<2$	28	32	32	32
4	Cyanide	Cool 4°C NaOH $pH>12$	14	32	32	—
5	Mercury	HNO $pH<2$ 0.05% $K_2Cr_2O_7$	28	6.8(12.2)	4.5(5.2)	10.5(25)
6	Mercury	HNO_3 $pH<2$ 0.05% $K_2Cr_2O_7$	28	32	11.1(15.6)	32
7	Mercury	HNO_3 $pH<2$	38†	5.3(8.9)	NV	32
8	Mercury	HNO_3 $pH<2$	13†	2.7(3.6)	NV	32
12	Ammonia	Cool 4°C Conc. H_2SO_4 $pH<2$	28	32	32	32
13	Fluoride	None	28	32	32	32
14	TKN	Cool 4°C Conc. H_2SO_4 $pH<2$	28	32	32	32
15	Nitrite plus nitrate	Cool 4°C Conc. H_2SO_4 $pH<2$	28	32	32	32
16	Organic carbon	Cool 4°C Conc. H_2SO_4 $pH<2$	28	32	32	32
17	Total phosphorus	Cool 4°C Conc. H_2SO_4 $pH<2$	28	32	32	32

(continued)

TABLE 5-1. (continued)

Experiment number	Parameter	Preservation technique	Proposed MHT's (days)	Experimentally determined MHT in matrices*		
				M1	M2	M3
18	Sulfide	Cool 4°C 2 mL/L of 2N zinc acetate	28	32	32	32
Drinking water parameters						
9	Nitrate	Conc. H_2SO_4 pH<2	14	16	16	16
10	Nitrate	Cool 4°C	2	16	16	16
11	Fluoride	None	30	32	32	32

* Experimentally determined MHT's are either 90 percent LCL of calculated MHT or maximum time of experiment. The values in parentheses are the calculated MHT's.

† MHT's for these preservation techniques were recommended by EPA (1979c).

NV: Calculated CT's and LCL's were not considered valid because the lack-of-fit variability was large.

Table 5-2. SUMMARY OF APPROXIMATE CONCENTRATION LEVELS (mg/L) STUDIED FOR EACH SAMPLE MATRIX

Parameter	Concentration levels sample matrices 1	2	3
Wastewater			
Phenols	0.200	0.050	0.400
Cyanide	0.13	0.25	—
Mercury	0.0025	0.0015	0.0055
Ammonia	9.5	1.8	0.60
Fluoride	7.50	35.0	15.5
TKN	130	6.5	1.4
Nitrite plus nitrate	28.0	7.7	47.0
Organic carbon	60.0	7.0	29.0
Total phosphorus	10.0	0.26	0.60
Sulfide	0.60	0.11	0.80
Drinking water			
Nitrate	1.00	9.20	4.5
Fluoride	0.90	0.37	1.60

TABLE 5-3. SUMMARY OF PERCENT STANDARD DEVIATIONS (CV'S) DERIVED FROM THE UNEXPLAINED VARIABILITY IN THE LACK-OF-LINEAR-FIT ANOVA TEST

Parameter	CV for sample matrices 1	 2	 3
Wastewater			
Phenols (Experiment 1)	14.4	18.3	36.7
(Experiment 2)	7.6	7.9	12.5
(Experiment 3)	15.6	20.7	44.8
Cyanide	5.0	15.5	--
Mercury (Experiment 5)	15.1	20.5	8.5
(Experiment 6)	18.8	7.7	5.9
(Experiment 7)	32.3	81.0	13.1
(Experiment 8)	16.1	58.6	17.4
Ammonia	8.0	10.1	7.2
Fluoride	9.1	1.8	3.0
TKN	7.7	10.9	9.8
Nitrite plus nitrate	9.6	8.4	1.4
Organic carbon	7.4	9.1	9.2
Total phosphorus	1.4	7.6	3.6
Sulfide	11.9	18.9	9.6
Drinking water			
Nitrate (Experiment 1)	16.3	7.0	7.5
(Experiment 2)	6.2	5.6	7.1
Fluoride	9.5	4.9	3.1

The phenols experiments using sulfuric acid as the preservative consistently exhibited higher CV's compared to the copper sulfate/phosphoric acid preservation experiment. The highest CV's were noted for Matrix 3 which also had the largest suspended solids content of the sample matrices obtained for the phenols experiments.

Large CV's for the mercury experiment occurred for samples preserved with only nitric acid and at concentrations less than 3 ug/L.

CYANIDE

Two apparently different types of problems occurred during the cyanide experiment on Matrices 2 and 3. The spike addition of sodium cyanide to the wastewater sample collected from the discharge of a primary batteries plant (SIC Code 3692) was lost within two days, while the background concentration of cyanide remained stable. The background concentration of cyanide in these samples was stable for unpreserved samples stored at 4°C for at least eight days.

The mechanisms for this phenomenon are not fully understood and were not investigated further at this time.

The spike addition of sodium cyanide to the wastewater sample collected from the discharge of an electroplating plant (SIC Code 3471) was not recovered and no cyanide was observed in the sample. It was discovered that the wastestream contained sulfur dioxide which was added to remove hexavalent chromium. Apparently the sulfur dioxide was converted to sulfide during the distillation procedure. Therefore, pretreatment of the sample to remove sulfide interferences prior to distilling as recommended was ineffective. Sulfide was observed in the distillate but not in the sample. Cyanide and sulfide combine to form thiocyanate and interfere with the analysis.

The use of a lead acetate scrubber to remove sulfide is a possible solution to the problems and is recommended in future research, but was not attempted in this study. Until further experimental support evidence is provided, the experimentally determined MHT of 32 days is apparently applicable only to certain sample types.

MERCURY

The known variability for mercury analyses, especially at sample concentrations less than 2.0 ug/L (Winter et al., 1977), and the effectiveness of 0.05 percent $K_2Cr_2O_7$ in samples acidified with HNO_3 to extract mercury from the walls of new, HNO_3-rinsed polyethylene Cubitainers®, were probably the main reasons a complete set of experimentally determined MHT's was not obtained.

The spiked concentration level for Matrix 3 was approximately 5 ug/L. At this higher concentration, precision is generally better. The results for Matrix 3 support long holding times for samples collected for mercury analysis preserved by the techniques used in Experiments 6, 7, and 8 (see Table 5-1). The best preservation and storage method, however, used 0.05 percent $K_2Cr_2O_7$ in samples acidified with nitric acid and stored in glass bottles (Experiment 6).

The data, when plotted for Experiment 5, exhibited positive slopes (concentration increases with time) for all three matrices. These results suggest container contamination problems. Analytical problems were unlikely because analyses for all experiments for a matrix were performed in the same sample lot. All plastic containers for Experiments 5 and 8 were new, unused polyethylene Cubitainers® rinsed with 1:1 HNO_3. When plotted for Experiment 8, the data exhibited either negative slopes or zero slope. The only difference between Experiments 5 and 8 was the use of 0.05 percent $K_2Cr_2O_7$ as part of the preservation. These results suggest that use of 0.05 percent $K_2Cr_2O_7$ assists in the extraction of mercury contamination adhered to the walls of plastic containers. Matsunaga et al. (1979) observed mercury contamination following washing with HNO_3 from both previously used and unused polyethylene bottles for samples stored for one month. The source of mercury contamination for new polyethylene bottles is not known; however, the use of plastic containers does not appear appropriate for monitoring mercury concentrations in discharge water when 0.05 percent $K_2Cr_2O_7$ is used as part of the preservation.

The experimental results and discussion of each parameter (Sections 5.1-5.10) include the following information:

1. MHT experiments performed.
2. Preservation techniques used.
3. Sources of variability specific to the method used.
4. Source of industrial discharges by SIC code or drinking waters by type of water source and treatment method sampled for each matrix (three per experiment).
5. Data summary, including mean and standard deviation, for unpreserved and preserved field samples collected for each matrix.
6. Approximate spiking levels for each matrix.
7. Raw data and summary statistics from time series tests for concentration changes with time, including the mean and standard deviation for each matrix and time combination; and the mean, standard deviation, and CV for the combined times for each matrix.
8. Estimates of analytical variability compared with literature values.
9. Evaluation of mixing effectiveness by examining the CV at Day 0 for each matrix.

The statistical evaluation summarizes the findings from the computer program used to specify the experimentally determined MHT. Also presented in this section for each parameter are graphs showing changes in concentration as a function of holding time for each experiment. Included on the figure for each matrix are:

1. A solid best-fit first-order curve indicating a significant trend for log concentration changes over time or a dashed curve indicating no significant trend (a slope not different than zero) at the 90 percent confidence level. Certain experiments were analyzed beyond the required time for the experiments, and these data were included in the computation. However, the time scale on the figures is for the required 16 or 32 days, depending on the experiment.

2. Lines representing plus and minus 10 percent deviations in concentration changes as determined from the intercept (at Day 0) of the best-fit regression line.

3. A vertical line representing the experimentally determined MHT for each matrix. The experimentally determined MHT is either 16 or 32 days in two cases: when the slope is not significantly different than zero (dashed curve), or when the 90 percent LCL of the calculated CT is greater than or equal to 16 or 32 days. The experimentally determined MHT is the 90 percent LCL in all otner cases.

The detailed methodology for the statistical interpretation of the data is discussed in Section 4.2.7; a complete documentation of the statistical program is provided in the Appendix.

5.1 PHENOLS

5.1.1 Experiments and Results

MHT experiments were performed for three preservation techniques of phenols in wastewaters as follows:

Experiment 1--Samples were stored in plastic containers at 4°C and preserved with sulfuric acid (H_2SO_4) to a pH of less than 2.0.

Experiment 2--Samples were stored in glass containers at 4°C and preserved with 1-gram per liter (g/L) copper sulfate and phosphoric acid (H_3PO_4) to a pH of less than 4.0.

Experiment 3--Samples were stored in glass containers at 4°C and preserved with sulfuric acid to a pH of less than 2.0.

The NPDES- and SDWA-approved 4-aminoantipyrine method (EPA Method 420.1) with distillation was used for analysis of phenol samples. The antipyrine dye produced was extracted from aqueous solution with chloroform, and the absorbance was measured at 460 nanometers (nm). Sufficient sample sizes for distillation were selected to ensure phenol concentrations within the working standard curve range of 0 to 200 ug/L of phenol.

Samples for the three matrices used in the three experiments were collected from three types of industrial dischargers:

Matrix 1--Petroleum Refining (SIC Code 2911);

Matrix 2--Blast Furnaces, Steel Works, and Rolling Mills (SIC Code 3312); and

Matrix 3--Broad Woven Fabric Mills, Cotton (SIC Code 2211).

Table 5-4 summarizes the data for unpreserved and preserved field samples analyzed at the start of the experiment (time equals zero). These analyses, at time equals zero, were performed within 48 hours after sample collection. Preservatives (other than chilling to 4°C) were added within 24 to 30 hours after sample collection during preparation of sample aliquots for holding time studies. Preserved sample concentrations were larger than unpreserved sample concentrations because of the time interval between preservation and analyses.

The large standard deviation in Experiment 2-Matrix 3 is possibly an effect of the preservative (1 g/L copper sulfate and H_3PO_4 to $pH < 4$) reacting with the particulates present in Matrix 3. Spiking levels were approximately 200, 44, and 400 ppb for each matrix, respectively, except that Experiment 1-Matrix 3 was spiked at approximately 200 ug/L.

Tables 5-5, 5-6, and 5-7 present the raw data and summary statistics for the phenol Experiments 1, 2, and 3, respectively. The CV's reported in these tables of the overall mean phenols concentrations determined for each time-sequenced analysis ranged from 4.84 to 23.1 percent. Lower CV's were observed for experiments performed for Matrix 1 and 2, while larger CV's occurred for Matrix 3 (Matrix 3 water had more particulate matter present). These CV's provide an estimate of analytical variablility; however, they can be inaccurate due to concentration changes with respect to time. A better estimate of day-to-day analytical variability from each matrix of the three experiments was determined from the lack-of-linear-fit terms of the individual analysis of variances (lack-of-fit ANOVA's) for the first-order model. The CV's calculated from the sum of squares of the differences (lack-fit term) were:

Experiment	Matrix	CV(%)
1	1	14.4
	2	18.3
	3	36.7
2	1	7.6
	2	7.9
	3	12.5
3	1	15.6
	2	20.7
	3	44.8

Analytical variability estimates were consistently smaller for Experiment 2 compared to Experiment 1 or 3 for the same matrix. These variabilities for Experiment 2 compared favorably with an EPA study (EPA, 1979a) for six laboratories with percent standard deviation ranges of 10.3 to 4.5 percent for a concentration range of 9.6 to 93.5 ug/L phenol, respectively. The estimated analytical variabilities for Matrices 1 and 2 of Experiments 1 and 3 are not unreasonably different than the reported analytical variability; however, it should be noted that the samples used for the EPA (1979a) study were probably not preserved using H_2SO_4. It is not certain why sulfuric acid preserved samples demonstrated larger estimated analytical variabilities. The estimated analytical variabilities were larger for Matrix 3 in all experiments apparently because of higher turbidities.

The variability for triplicate samples analyzed at Day 0 were used to evaluate the homogeneity of the dispensed samples. The percent standard deviations at Day 0 for each matrix and experiment indicating acceptable mixing were:

Experiment	Matrix	CV (%)
1	1	6.1
	2	2.5
	3	11.4
2	1	4.8
	2	19.2
	3	12.8
3	1	4.8
	2	2.0
	3	0.99

5.1.2 Statistical Evaluation

The first-order model exhibited the best distributional characteristics (normality and homogeneity) compared to the zero-order model for the standardized residuals of the overall two-way ANOVA's. Results from the individual lack-of-fit ANOVA's (for each matrix) indicated that the log-concentration-versus-time trends for each matrix were not best explained by linear fits. In other words, day-to-day variability was greater than within-day variability. Since the variability associated with the lack of linear fit for Experiment 2 and Matrices 1 and 2 of Experiment 1 and 3 compared favorably to expected analytical variability, the calculated CT's and LCL's for those matrices were assumed valid. Duncan's multiple range test for Matrix 3 of Experiments 2 and 3 was examined for randomness. It appears that the larger lack of fit from Matrix 3 results from turbidity effects, and probably the calculated CT's and LCL's are valid. Except for Matrix 3 of Experiments 1 and 3, the precision of the method allowed a 10 percent concentration change to be significant. At the 90 percent confidence level, significant percent changes in concentration from the intercept of the best-fit regression line were:

Experiment	Matrix	% Change
1	1	4.5
	2	6.9
	3	14.7
2	1	3.2
	2	4.9
	3	6.5
3	1	5.2
	2	6.5
	3	13.8

Figures 5-1, 5-2, and 5-3 show the statistical results for Experiments 1, 2, and 3, respectively. The calculated CT's and 90 percent LCL's were:

Experiment	Matrix	CT (days)	LCL (days)
1	1	32	32
	2	32	32
	3	32	32
2	1	16	16
	2	7.9	4.9
	3	4.2	3.1
3	1	32	32
	2	32	32
	3	32	32

The slopes for Experiments 1 and 3 and Matrix 1 of Experiment 2 were not significantly different (insignificant slope on figures) than a slope of zero (no change in concentration with time). Therefore, the experimentally determined MHT for phenols experiments using sulfuric acid as the preservative is 32 days, while the experimentally determined MHT is low (three days) for preservation with H_3PO_3 and copper sulfate. These results compare with Carter and Huston (1978) who showed that preserving with H_2SO_4 to pH less than 2.0 effectively reduces biologically induced degradation of phenolic compounds.

Table 5-4. Phenols (ug/L) Results for Samples Analyzed at Time = 0.

MATRIX	EXPERIMENT	SAMPLE TYPE	MEAN (MG/L)	STD.DEV. (MG/L)
1	1	USUP	15.75	0.72
1	1	USP	29.26	1.39
1	2	USUP	17.34	1.40
1	2	USP	29.01	2.45
1	3	USUP	18.35	2.80
1	3	USP	27.58	0.77
2	1	USUP	9.81	3.63
2	1	USP	17.99	1.51
2	2	USUP	5.23	0.78
2	2	USP	4.71	0.64
2	3	USUP	8.74	0.79
2	3	USP	22.53	1.05
3	1	USUP	2.92	3.62
3	1	USP	18.14	9.86
3	2	USUP	7.56	1.23
3	2	USP	72.94	47.34
3	3	USUP	5.25	0.80
3	3	USP	35.16	5.32

USUP = UNSPIKED,UNPRESERVED
USP = UNSPIKED,PRESERVED

Table 5-5. Phenols (ug/L) Raw Data and Summary Statistics for Experiment 1.

TIME	MATRIX 1			MATRIX 2			MATRIX 3		
	DATA	MEAN	S.D.	DATA	MEAN	S.D.	DATA	MEAN	S.D.
0	221.6	229.5	13.89	57.40	56.98	1.424	209.4	194.3	22.09
	221.3			55.40			204.6		
	245.5			58.15			169.0		
2	181.7	189.7	10.63	41.11	49.48	7.290	222.7	206.3	28.35
	185.6			54.41			222.7		
	201.8			52.93			173.6		
4	195.2	194.4	4.395	59.68	56.60	5.330	170.0	165.2	6.223
	198.3			59.68			158.1		
	189.7			50.45			167.4		
8	183.8	190.7	7.963	52.01	50.77	1.238	121.9	126.6	20.34
	199.4			49.53			109.0		
	189.0			50.77			148.9		
16	178.9	186.9	7.027	48.04	44.38	4.931	153.0	148.3	54.12
	189.7			46.34			92.02		
	192.1			38.78			200.0		
32	203.4	201.5	1.777	50.07	53.80	3.372	140.7	186.6	40.63
	199.9			54.73			218.0		
	201.2			56.61			201.2		
	MEAN	S.D.	C.V.	MEAN	S.D	C.V.	MEAN	S.D.	C.V.
TOTAL	198.8	16.55	8.323	52.00	5.877	11.30	171.2	39.34	22.98

S.D. = Standard Deviation
C.V. = Coefficient of Variation (percent)
CONC = Concentration in ug/L

Table 5-6. Phenols (ug/L) Raw Data and Summary
Statistics for Experiment 2.

TIME	MATRIX 1 DATA	MATRIX 1 MEAN	MATRIX 1 S.D.	MATRIX 2 DATA	MATRIX 2 MEAN	MATRIX 2 S.D.	MATRIX 3 DATA	MATRIX 3 MEAN	MATRIX 3 S.D.
0	283.6 299.8 312.3	298.5	14.40	41.50 60.92 49.90	50.77	9.743	499.1 433.6 386.9	439.9	56.35
1				47.77 51.95 50.52	50.08	2.121	399.2 341.8 386.9	376.0	30.21
2	283.5 268.8 278.3	276.9	7.467	53.67 53.17 57.23	54.69	2.213	308.6 349.1 379.6	345.8	35.63
4	286.6 288.6 282.5	285.9	3.120	49.47 45.79 50.95	48.74	2.655	316.9 358.7 345.7	340.4	21.38
8	270.1 272.3 275.1	272.5	2.508	47.06 48.54 46.56	47.39	1.030	311.4 339.9 330.8	327.4	14.55
16	305.6 300.1 304.2	303.3	2.880	38.53 43.41 42.96	41.64	2.699	214.6 299.8 288.4	267.6	46.24
	MEAN	S.D.	C.V.	MEAN	S.D	C.V.	MEAN	S.D.	C.V.
TOTAL	287.4	13.92	4.843	48.88	5.530	11.31	349.5	61.75	17.67

Blanks in Table Indicate Samples were not Analyzed on that day
S.D. = Standard Deviation
C.V. = Coefficient of Variation (percent)
CONC = Concentration in ug/L

Table 5-7. Phenols (ug/L) Raw Data and Summary Statistics for Experiment 3.

TIME	MATRIX 1 DATA	 MEAN	 S.D.	MATRIX 2 DATA	 MEAN	 S.D.	MATRIX 3 DATA	 MEAN	 S.D.
0	346.7	330.1	15.87	74.49	74.59	1.455	433.6	438.1	4.339
	315.1			73.19			438.5		
	328.6			76.10			442.2		
2	287.2	262.4	29.34	55.69	60.44	8.232	345.3	336.1	25.39
	230.0			55.69			307.3		
	270.0			69.95			355.5		
4	268.9	273.3	13.58	61.94	65.57	3.210	316.9	309.5	26.30
	262.4			66.72			280.3		
	288.5			68.04			331.3		
8	276.6	283.7	6.470	61.18	62.42	1.084	201.7	218.8	20.56
	285.2			62.92			213.3		
	289.2			63.17			241.6		
16	272.9	285.3	13.73	68.12	56.92	10.24	212.2	283.1	64.18
	283.0			48.04			299.8		
	300.1			54.60			337.2		
32	300.9	300.1	1.072	68.70	71.93	2.913	303.0	332.9	39.02
	298.9			72.74			377.0		
	300.4			74.35			318.6		
	MEAN	S.D.	C.V.	MEAN	S.D	C.V.	MEAN	S.D.	C.V.
TOTAL	289.1	26.00	8.990	65.31	7.996	12.24	319.7	73.99	23.14

S.D. = Standard Deviation
C.V. = Coefficient of Variation (percent)
Conc = Concentration in ug/L

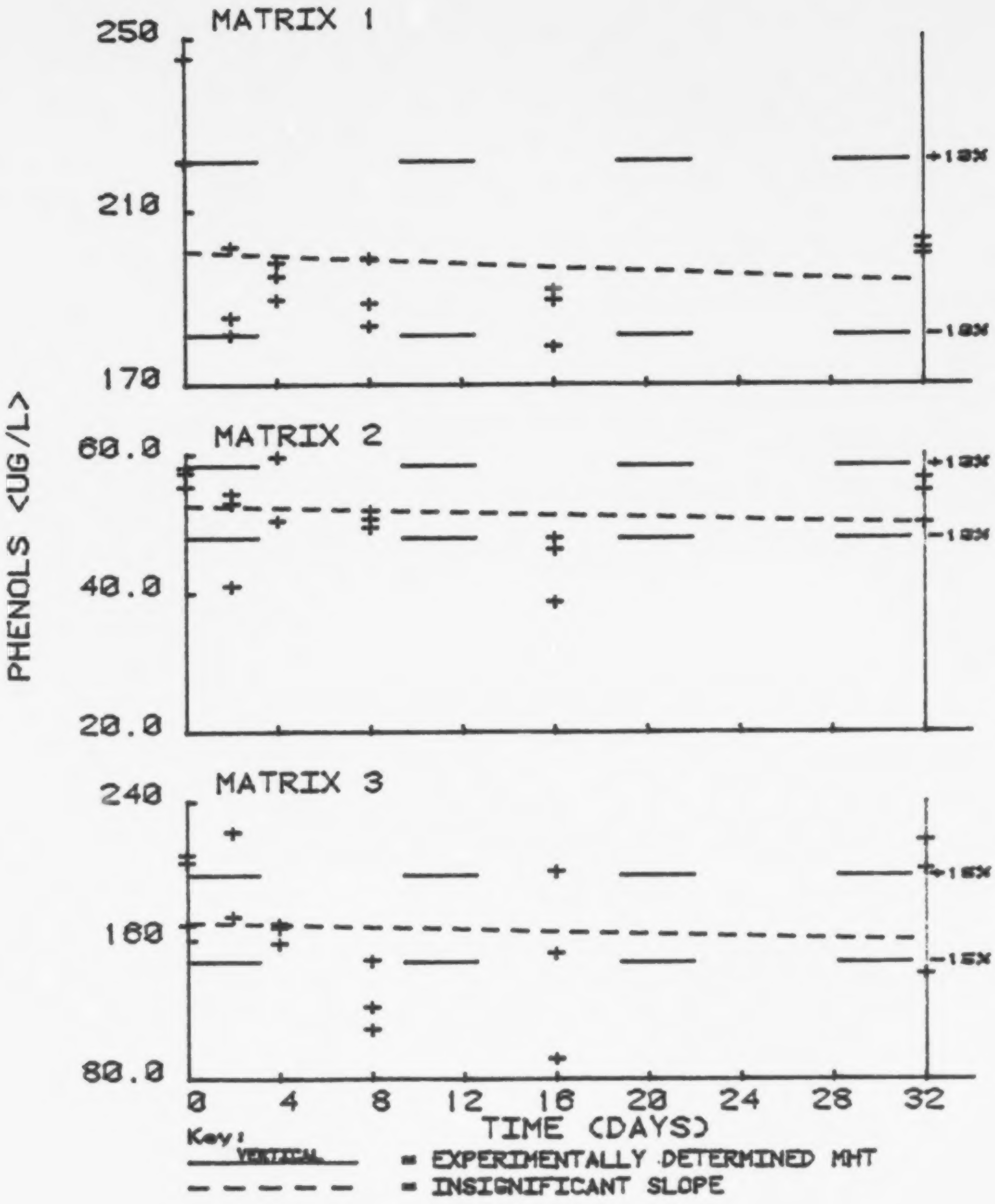


Figure 5-1. STATISTICAL RESULTS OF EXPERIMENT 1 FOR PHENOLS

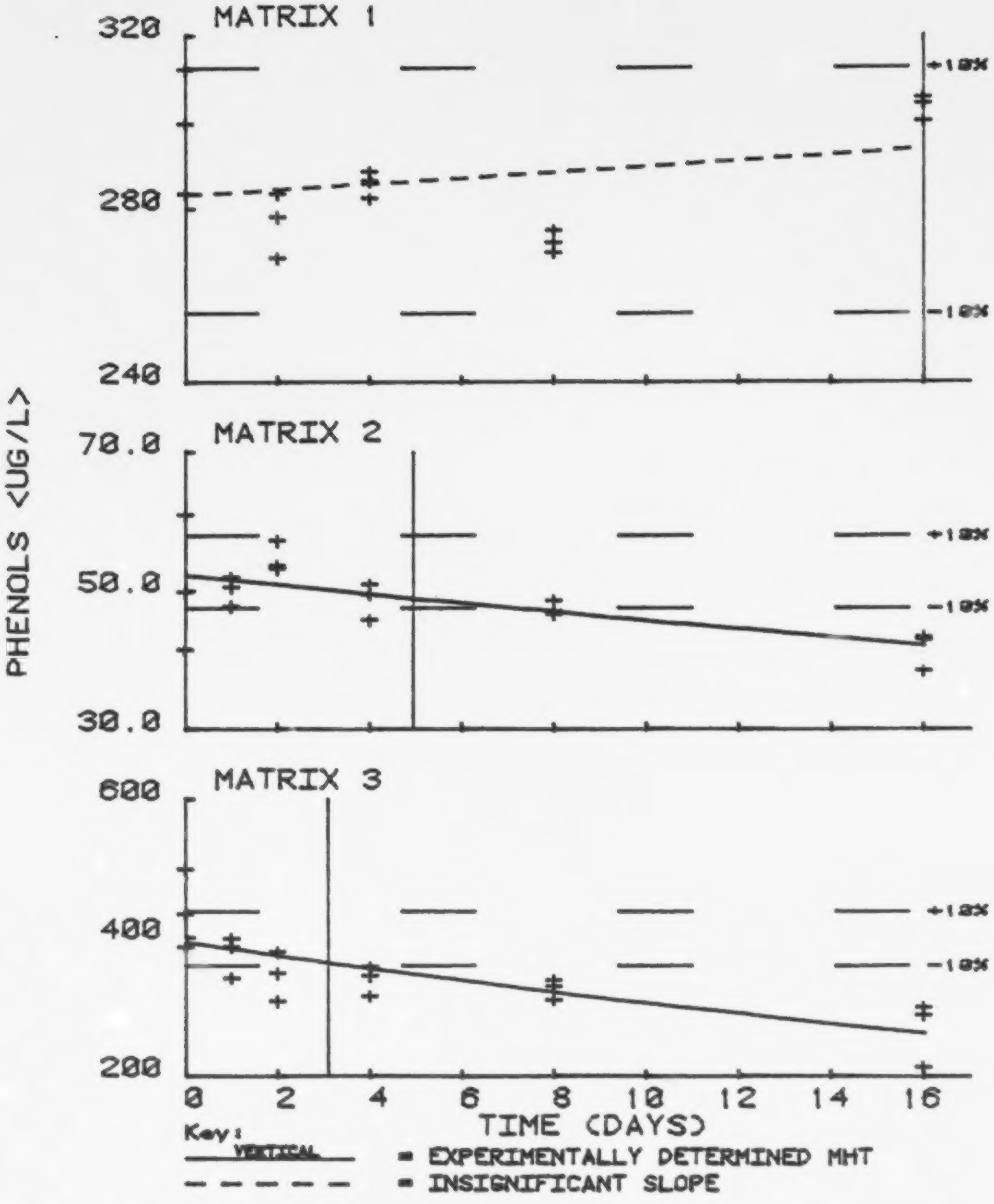


Figure 5-2. STATISTICAL RESULTS OF
EXPERIMENT 2 FOR PHENOLS

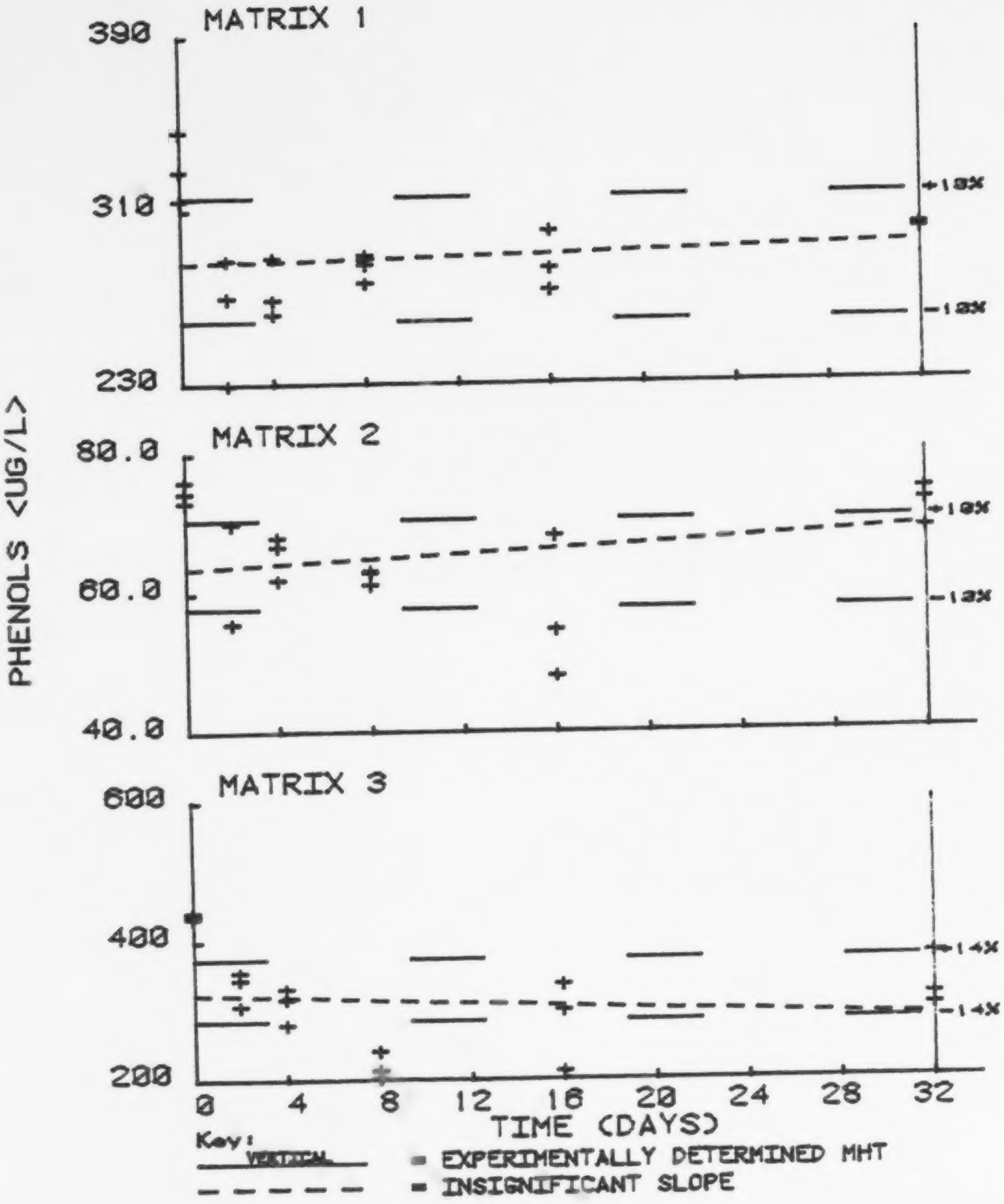


Figure 5-3. STATISTICAL RESULTS OF EXPERIMENT 3 FOR PHENOLS

5.2 CYANIDE

5.2.1 Experiments and Results

One MHT experiment was performed for cyanide analysis in wastewater. The sample preservation technique included addition of ascorbic acid when chlorine is present, pH adjustment to a pH greater than 12.0 with sodium hydroxide, and storage at 4°C in plastic containers. Cyanide was measured by the spectrophotometric EPA Method 335.2.

Samples were collected for three matrices from three types of industrial dischargers:

Matrix 1--Blast Furnaces, Steel Works, and Rolling Mills (SIC Code 3312);

Matrix 2--Primary Batteries, Dry and Wet (SIC Code 3692); and

Matrix 3--Electroplating, Plating, Polishing, Anodizing, and Coloring (SIC Code 3471).

Table 5-8 summarizes the data for unpreserved and preserved field samples analyzed at time equals zero. These analyses, at time equals zero, were performed within 48 hours after sample collection. Preservatives (other than chilling to 4°C) were added within 24 to 30 hours after sample collection during preparation of sample aliquots for holding time studies. There were no significant differences between unpreserved and preserved samples. Spiking levels were approximately 0.11, 0.20, and 0.06 mg/L for each matrix, respectively.

Table 5-9 presents the raw data and summary statistics for the cyanide experiment. Summary statistics are not available for Matrix 3 in Table 5-7 for the following reasons:

1. Sulfur dioxide was added to the wastestream before the sampling point.

2. During the reflux distillation analysis step, sulfide was formed and distilled over into the alkaline distillate.

3. Thiocyanate is formed when cyanide and sulfide are present in alkaline conditions; therefore, cyanide measurements were not valid as 0 mg/L with 0 percent recoveries.

All sodium cyanide spiked in Matrix 2 was lost by Day 2; however, background levels remained relatively constant for the 32 days. The reason for this phenomenon is not fully understood. Statistical information is reported for Matrix 2 without time equals Day 0. The CV's of the overall cyanide concentrations observed for the combined times for each matrix were 4.52 and 12.58, respectively. An estimate of day-to-day analytical variability for each matrix was determined from the lack-fit term of the individual lack-of-fit ANOVA's for the first-order model. The CV's from the lack-fit terms were 5.0 and 15.5 percent, respectively.

These lack-of-fit variabilities compared favorably with an EPA study (EPA, 1979a) for a single laboratory which reported percent standard deviation ranges of 5.4 to 15.2 for a concentration range of 0.06 to 0.62 mg/L cyanide. It is not known whether the variability for the single laboratory was determined on a single day or from several days of analyses in that EPA study. The CV's at Day 0 for each matrix were 2.4 and 3.8 percent, indicating well-mixed samples.

5.2.2 Statistical Evaluation

The first-order model exhibited the best distributional characteristics (normality and homogeneity) compared to the zero-order model for the standardized residuals of the overall two-way ANOVA. Results from the individual lack-of-fit ANOVA's (for each matrix) indicated that the log-concentration-versus-time trends for each matrix were not best explained by linear fits. In other words, day-to-day analytical variability was greater than within-day variability. Since the estimated analytical variability associated with the lack of linear fit compared favorably with the expected analytical variability, the calculated CT's and LCL's can be assumed valid. Calculated CT's and LCL's were not valid for Matrix 2 when Day 0 is included in the evaluation because the lack-of-fit variability (55.5 percent) suggested a higher-order model was needed. The experimentally determined MHT for Matrix 2 with Day 0 included would have to be estimated as less than two days.

Figure 5-4 presents the statistical results for Matrices 1 and 2. The slopes for the log-concentration-versus-time regression lines were not significantly different than zero (no change in concentration with time). The precision of the method allowed a 10 percent change in concentration to be significant. The significant (90 percent confidence level) percent changes in concentration from the intercept of the best-fit regression line were 2.7 and 9.1 percent for Matrices 1 and 2.

5.2.3 Discussion

The spike addition of sodium cyanide to a wastewater sample collected from the discharge of a primary batteries plant (SIC Code 3692) was lost within two days, while background concentrations of cyanide remained stable. The background concentrations were stable also for unpreserved samples stored at 4°C for at least eight days. The currently approved analytical method for cyanide (EPA, 1979a and 1979c) cannot accurately determine cyanide concentrations in wastewater samples containing sulfur compounds which can be converted to sulfide during the distillation procedure. Cyanide and sulfide combine to form thiocyanate and cannot be measured by the analytical method for cyanide. EPA has revised the analytical method for cyanide to include a lead acetate scrubber to remove sulfide formed during the distillation step. These two main problems encountered with Matrices 2 and 3 during this experiment suggest the need for further study.

Until further experimental support evidence is provided, the experimentally determined MHT of 32 days is applicable only to certain sample types. In fact, using the currently approved analytical method, the concentration of cyanide in certain sample types cannot be accurately determined because of the presence of certain sulfur-containing compounds.

Table 5-8. Cyanide (mg/L) Results for Samples Analyzed at Time = 0.

MATRIX	EXPERIMENT	SAMPLE TYPE	MEAN (MG/L)	STD.DEV. (MG/L)
1	4	USUP	0.004	0.002
1	4	USP	0.003	0.003
2	4	USUP	0.110	0.012
2	4	USP	0.098	0.018
3	4	USUP	0.007	0.002
3	4	USP	0.008	0.006

USUP = UNSPIKED,UNPRESERVED
USP = UNSPIKED,PRESERVED

Table 5-9. Cyanide (mg/L) Raw Data and Summary Statistics for Experiment 4.

TIME		MATRIX 1			MATRIX 2			MATRIX 3	
	DATA	MEAN	S.D.	DATA	MEAN	S.D.	DATA	MEAN	S.D.
0	0.139 0.133 0.134	0.135	0.003	0.242 0.225 0.230	0.233	0.009			
2	0.122 0.132 0.132	0.129	0.006	0.112 0.095 0.093	0.100	0.010			
4	0.116 0.130 0.136	0.128	0.010	0.110 0.138 0.128	0.125	0.014			
8	0.128 0.125 0.131	0.128	0.003	0.123 0.096 0.114	0.111	0.014			
16	0.129 0.122 0.129	0.127	0.004	0.123 0.090 0.127	0.113	0.020			
32	0.130 0.128 0.139	0.132	0.006	0.112 0.117 0.122	0.117	0.005			
	MEAN	S.D.	C.V.	MEAN	S.D	C.V.	MEAN	S.D.	C.V.
TOTAL	0.130	0.006	4.517	0.133	0.048	35.73	318.5		

Blanks in Table Indicate Samples were not Analyzed on that day
S.D. = Standard Deviation
C.V. = Coefficient of Variation (percent)
CONC = Concentration in mg/L

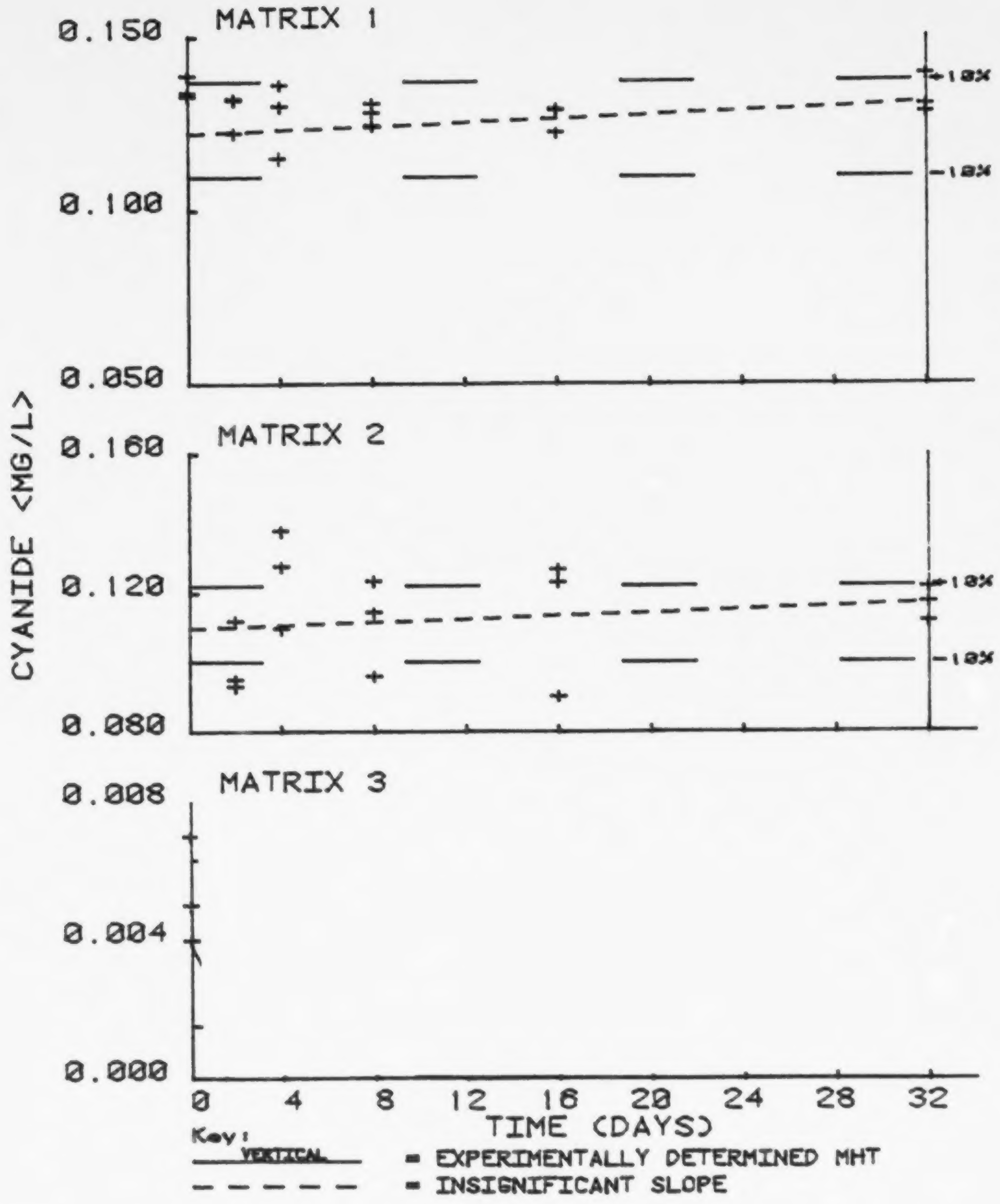


Figure 5-4. STATISTICAL RESULTS OF EXPERIMENT 4 FOR CYANIDE

5.3 MERCURY

5.3.1 Experiments and Results

Four MHT experiments were performed for two preservation techniques with sample storage in both glass and plastic containers as follows:

Experiment 5--Samples were stored in plastic and preserved with HNO_3 to a pH of less than 2.0 and 0.05 percent $K_2Cr_2O_7$.

Experiment 6--Samples were stored in glass and preserved with HNO_3 to a pH of less than 2.0 and 0.05 percent $K_2Cr_2O_7$.

Experiment 7--Samples were stored in glass containers and preserved only with HNO_3 to a pH of less than 2.0.

Experiment 8--Samples were stored in plastic containers and preserved only with HNO_3 to a pH of less than 2.0

All samples were stored at room temperature. The cold vapor technique was used for the analysis of mercury samples using the Perkin Elmer (1981) MHS-20 system. Instead of measuring the mass of mercury in the bottle, 25 mL of the digested and treated sample was used for analysis in the MHS-20 system.

Samples for the three wastewater matrices used in the four experiments were collected from three types of industrial dischargers:

Matrix 1--Alkalies and Chlorine (SIC Code 2812);

Matrix 2--Coating, Engraving, and Allies Services, NEC (SIC Code 3479); and

Matrix 3--Industrial Organic Chemicals, NEC (SIC Code 2869).

Each collected sample was mixed and dispensed on the same day so analyses occurred on the same day for all four experiments. Table 5-10 summarizes the data for the unpreserved and preserved field samples from each experiment analyzed at time equals Day 0. These analyses, at time equals zero, were performed within 48 hours after sample collection. Preservatives (other than chilling to 4°C) were added within 24 to 30 hours after sample collection during preparation of sample aliquots for holding time studies. These analyses, at time equals zero, were performed within 48 hours after sample collection. Preservatives (other than chilling to 4°C) were added within 24 to 30 hours after sample collection during preparation of sample aliquots for holding time studies. The background concentrations for the three industrial wastes were each below 1.0 ug/L. This concentration level is usually associated with more variability than higher mercury concentrations (EPA, 1979a). No significant difference is apparent between unpreserved and preserved field samples for each matrix/experiment, except for an apparent contamination of 0.27 ug/L mercury caused by the addition of potassium dichromate in Matrix 2 for Experiments 5 and 6. This possible contamination problem did not require reanalysis of the experiments since the purpose was to monitor the change of concentration with time.

Spiking levels were approximately 2.0, 1.0, and 5.0 ug/L for each matrix, respectively, for the four experiments. Tables 5-11 through 5-14 present the raw data and summary statistics for mercury Experiments 5 through 8, respectively. The CV's of the overall concentrations observed for the combined times of each matrix ranged from 8.35 to 51.3 percent for all experiments. Generally, these CV's improved with increasing analyte concentration as expected since analytical precision is improved at higher concentrations.

An estimate of day-to-day analytical variability for each matrix of the four experiments was determined from the lack-fit term of the individual lack-of-fit ANOVA's for the first-order model. The sum of squares of the error associated with the lack-fit term of each ANOVA was converted to a CV (see Equations 4 and 5, Section 4.2.7). These lack-of-fit CV's for each matrix of each experiment were:

Experiment	Matrix	CV(%)
5	1	15.1
	2	20.5
	3	8.5
6	1	18.8
	2	7.7
	3	5.9
7	1	32.3
	2	81
	3	13.1
8	1	16.1
	2	58.6
	3	17.4

These estimated analytical variabilities compared favorably with a joint EPA/ASTM (EPA, 1979a) interlaboratory study where the CV's for mean values ranging from 0.709 to 9.10 ug/L ranged from 29 to 55 percent. The only exceptions were in Matrix 2 of Experiments 7 and 8. Results from a single laboratory on a single day clearly indicate the effect of concentration on precision. The CV's for Ohio River water ranged from 46 to 2 percent for a concentration range of 0.35 to 4.35, respectively (EPA, 1979a). Other EPA studies (EPA, 1977) have shown the relatively poor precision for this mercury method.

The variability for triplicate samples at Day 0 was used to evaluate the homogeneity of the dispensed samples. The CV's at Day 0 for each matrix (100 x std. dev./mean) ranged from 2.8 to 18 percent, indicating well-mixed samples. The CV at Day 0 for each matrix were:

Experiment	Matrix	CV(%)
5	1	18
	2	3.4
	3	4.9
6	1	6.6
	2	7.5
	3	15
7	1	3.7
	2	9.7
	3	9.9
8	1	5.0
	2	7.2
	3	2.8

5.3.2 Statistical Evaluation

Generally, the first-order model exhibited the best distributional characteristics (normality and homogeneity) compared to the zero-order model for the standardized residuals of the overall two-way ANOVA for each experiment. Results from most of the individual lack-of-fit ANOVA's (for each matrix of each experiment) indicated that the log-concentration-versus-time trends for each matrix were not best explained by linear fits. Since the estimated analytical variability associated with the lack of linear fit compared favorably to the expected analytical variability, the calculated CT's and LCL's were assumed valid except for Matrix 2 in Experiments 7 and 8. At the 90 percent significance level, significant percent changes in concentration from the intercept of the best-fit regression line were:

Experiment	Matrix	% Change
5	1	7.0
	2	4.9
	3	4.4
6	1	6.1
	2	3.7
	3	4.7
7	1	13.1
	2	51.2
	3	5.2

Experiment	Matrix	% Change
8	1	7.5
	2	22.2
	3	5.3

Figures 5-5 through 5-8 show the results for Experiments 5 through 8, respectively. Slopes that were not significantly different than a slope of zero are represented by dashed lines, while solid lines represent slopes significantly different than zero. The calculated CT's and LCL's were:

Experiment	Matrix	CT (days)	LCL (days)
5	1	12.2	6.8
	2	5.2	4.5
	3	24.8	10.5
6	1	32	32
	2	15.6	11.1
	3	32	32
7	1	8.9	5.3
	2	NV*	NV
	3	32	32
8	1	3.6	2.7
	2	NV	NV
	3	16	16

* NV = not considered statistically valid.

The calculated CT's and LCL's were not considered statistically valid (NV) for Matrix 2 of Experiments 7 and 8. Slopes that were not significantly different than zero (no concentration change with time) were observed for Matrix 3 of Experiments 6, 7, and 8.

5.3.3 Discussion

The known variability for mercury analyses, especially at sample concentrations less than 2.0 ug/L, and the effectiveness of 0.05 percent $K_2Cr_2O_7$ in samples acidified with HNO_3 to extract mercury from the walls of new, HNO_3-rinsed polyethylene Cubitainers®, were the main reasons the experimentally determined MHT's did not exceed the proposed or recommended values.

The spiked concentration level for Matrix 3 was approximately 5 ug/L. At this higher concentration, precision is generally better. The results for Matrix 3 support long holding times for samples collected for mercury analysis preserved by the techniques used in Experiments 6, 7, and 8. The best preservation and storage method, however, used HNO_3 and $K_2Cr_2O_7$ with storage in glass bottles (Experiment 6) because Matrices 1 and 2 provided better calculated CT's and LCL's compared to the other experiments.

The data, when plotted for Experiment 5, exhibited positive slopes (concentration increases with time) for all three matrices. These results suggest container contamination problems. Analytical problems were unlikely because analyses for all experiments for each matrix were performed in the same sample lot. All plastic containers for Experiments 5 and 8 were unused polyethylene Cubitainers® rinsed with 1:1 HNO_3. When plotted for Experiment 8, data exhibited either negative slopes or no slope. The only difference between Experiments 5 and 8 was the use of 0.05 percent $K_2Cr_2O_7$ as part of the preservation. These results suggest that use of 0.05 percent $K_2Cr_2O_7$ assists in the extraction of mercury contamination adhered to the walls of plastic containers. Matsunaga *et al.* (1979) observed mercury contamination following washing with HNO_3 from both previously used and unused polyethylene bottles for samples stored for one month. The source of mercury contamination for new polyethylene bottles is not known; however, the use of plastic containers does not appear appropriate for monitoring mercury concentrations in discharge water.

TABLE 5-8. Mercury (ug/L) Results for Samples Analyzed at Time = 0.

MATRIX	EXPERIMENT	SAMPLE TYPE	MEAN (MG/L)	STD.DEV. (MG/L)
1	5	USUP	0.404	0.155
1	5	USP	0.436	0.028
1	6	USUP	0.403	0.056
1	6	USP	0.387	0.497
1	7	USUP	0.313	0.209
1	7	USP	0.362	0.238
1	8	USUP	0.533	0.028
1	8	USP	0.631	0.028
2	5	USUP	0.100	0.000
2	5	USP	0.381	0.065
2	6	USUP	0.100	0.000
2	6	USP	0.368	0.051
2	7	USUP	0.100	0.000
2	7	USP	0.100	0.000
2	8	USUP	0.174	0.129
2	8	USP	0.100	0.000
3	5	USUP	0.692	0.053
3	5	USP	0.722	0.052
3	6	USUP	0.339	0.290
3	6	USP	0.319	0.272
3	7	USUP	0.384	0.305
3	7	USP	0.391	0.207
3	8	USUP	0.575	0.201
3	8	USP	0.707	0.387

USUP = UNSPIKED,UNPRESERVED
USP = UNSPIKED,PRESERVED

Table 5-11. Mercury (ug/L) Raw Data and Summary Statistics for Experiment 5.

TIME	MATRIX 1			MATRIX 2			MATRIX 3		
	DATA	MEAN	S.D.	DATA	MEAN	S.D.	DATA	MEAN	S.D.
0	2.254	2.544	0.457	1.305	1.280	0.043	5.565	5.413	0.263
	2.307			1.305			5.110		
	3.070			1.230			5.565		
2	2.816	2.636	0.168	1.326	1.380	0.047	4.877	5.008	0.226
	2.608			1.407			4.877		
	2.484			1.407			5.269		
4	2.123	2.324	0.263	1.878	1.810	0.164	5.332	5.200	0.228
	2.229			1.623			4.937		
	2.621			1.929			5.332		
8	3.161	3.007	0.134	1.584	1.569	0.026	5.392	5.648	0.256
	2.929			1.539			5.648		
	2.929			1.584			5.905		
16	3.166	2.716	0.409	1.788	1.657	0.113	5.517	5.902	0.575
	2.366			1.592			6.562		
	2.615			1.592			5.625		
32	3.314	3.262	0.186	2.217	2.447	0.311	6.581	5.828	0.731
	3.417			2.801			5.780		
	3.056			2.323			5.122		
	MEAN	S.D.	C.V.	MEAN	S.D	C.V.	MEAN	S.D.	C.V.
TOTAL	2.748	0.402	14.64	1.691	0.412	24.37	5.500	0.491	8.925

Blanks in Table Indicate Samples were not Analyzed on that day
S.D. = Standard Deviation
C.V. = Coefficient of Variation (percent)
CONC = Concentration in ug/L

Table 5-12. Mercury (ug/L) Raw Data and Summary Statistics for Experiment 6.

TIME		MATRIX 1			MATRIX 2			MATRIX 3	
	DATA	MEAN	S.D.	DATA	MEAN	S.D.	DATA	MEAN	S.D.
0	2.576	2.649	0.175	1.686	1.557	0.117	6.386	5.429	0.832
	2.522			1.531			5.019		
	2.849			1.455			4.882		
2	2.195	2.209	0.024	1.734	1.639	0.103	5.820	5.209	0.610
	2.236			1.652			5.207		
	2.195			1.529			4.600		
4	2.406	2.418	0.090	1.521	1.640	0.128	6.015	5.559	0.395
	2.335			1.776			5.332		
	2.514			1.623			5.332		
8	2.697	2.658	0.039	1.548	1.546	0.006	5.392	5.562	0.148
	2.619			1.550			5.648		
	2.658			1.539			5.648		
16	1.970	2.184	0.223	1.658	1.636	0.038	5.303	5.467	0.479
	2.416			1.658			5.091		
	2.168			1.592			6.007		
32	2.954	2.783	0.180	1.798	1.937	0.121	5.133	5.126	0.006
	2.594			2.007			5.122		
	2.800			2.007			5.122		
	MEAN	S.D.	C.V.	MEAN	S.D	C.V.	MEAN	S.D.	C.V.
TOTAL	2.484	0.265	10.68	1.659	0.157	9.468	5.392	0.450	8.352

Blanks in Table Indicate Samples were not Analyzed on that day
S.D. = Standard Deviation
C.V. = Coefficient of Variation (percent)
CONC = Concentration in ug/L

Table 5-13. Mercury (ug/L) Raw Data and Summary Statistics for Experiment 7.

TIME	MATRIX 1 DATA	MATRIX 1 MEAN	MATRIX 1 S.D.	MATRIX 2 DATA	MATRIX 2 MEAN	MATRIX 2 S.D.	MATRIX 3 DATA	MATRIX 3 MEAN	MATRIX 3 S.D.
0	2.522	2.577	0.094	0.833	0.752	0.073	4.973	5.611	0.554
	2.685			0.694			5.884		
	2.522			0.728			5.975		
2	1.825	1.976	0.144	0.843	0.856	0.023	4.619	4.684	0.170
	1.989			0.883			4.877		
	2.113			0.843			4.555		
4	1.566	1.453	0.142	0.050	0.151	0.101	5.207	4.984	0.334
	1.498			0.152			5.146		
	1.294			0.253			4.600		
8	2.116	1.818	0.361	0.738	0.753	0.026	5.562	5.449	0.354
	1.921			0.782			5.733		
	1.416			0.738			5.052		
16	1.675	1.724	0.049	0.532	0.532	0.201	5.843	5.379	0.547
	1.724			0.331			5.517		
	1.773			0.732			4.776		
32	0.962	1.318	0.574	1.538	1.280	0.258	4.830	4.928	0.169
	1.013			1.279			4.830		
	1.980			1.023			5.122		
	MEAN	S.D.	C.V.	MEAN	S.D	C.V.	MEAN	S.D.	C.V.
TOTAL	1.811	0.485	26.79	0.721	0.370	51.32	5.172	0.469	9.066

Blanks in Table Indicate Samples were not Analyzed on that day
S.D. = Standard Deviation
C.V. = Coefficient of Variation (percent)
CONC = Concentration in ug/L

Table 5-14. Mercury (ug/L) Raw Data and Summary
Statistics for Experiment 8.

TIME	MATRIX 1			MATRIX 2			MATRIX 3		
	DATA	MEAN	S.D.	DATA	MEAN	S.D.	DATA	MEAN	S.D.
0	2.904 2.740 2.631	2.758	0.138	0.728 0.694 0.798	0.740	0.053	4.973 5.201 5.246	5.140	0.146
1	2.774 2.899 2.816	2.830	0.064	0.709 0.746 1.316	0.924	0.340	5.299 5.198 5.147	5.215	0.078
2	2.608 1.907 2.691	2.402	0.431	0.484 0.603 0.723	0.603	0.119	4.683 4.428 4.942	4.684	0.257
4	2.406 2.087 1.704	2.066	0.352	0.253 0.810 0.303	0.455	0.308	4.104 3.994 4.214	4.104	0.110
8	1.883 1.921 2.348	2.051	0.258	0.384 0.428 0.428	0.414	0.025	5.052 4.883 5.137	5.024	0.129
16	1.871 1.528 1.773	1.724	0.177	0.666 0.599 0.599	0.621	0.039	5.357 4.985 4.776	5.039	0.294
	MEAN	S.D.	C.V.	MEAN	S.D	C.V.	MEAN	S.D.	C.V.
TOTAL	2.305	0.467	20.26	0.626	0.241	38.51	4.868	0.421	8.651

Blanks in Table Indicate Samples were not Analyzed on that day
S.D. = Standard Deviation
C.V. = Coefficient of Variation (percent)
CONC = Concentration in ug/L

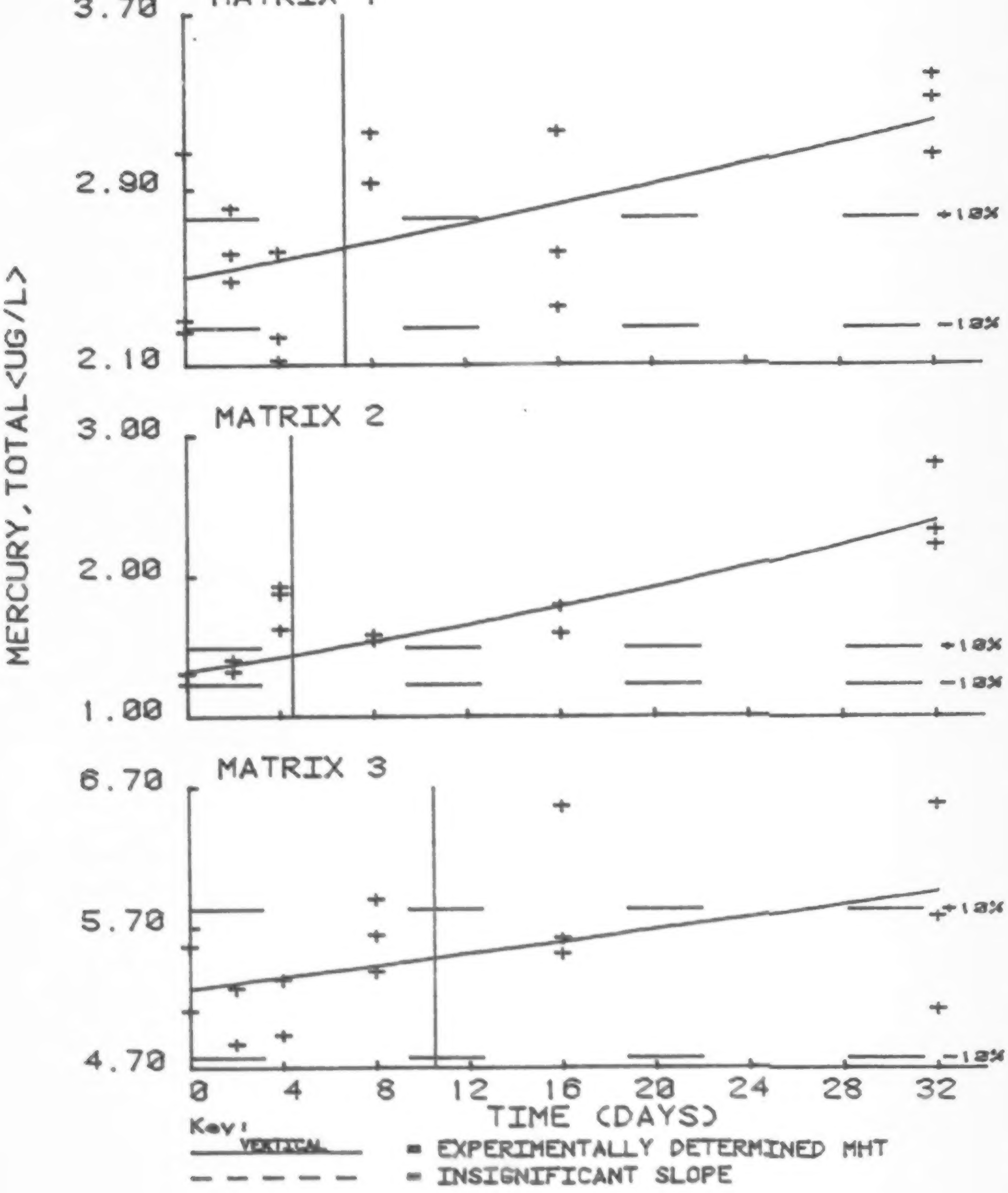


Figure 5-5. STATISTICAL RESULTS OF EXPERIMENT 5 FOR MERCURY

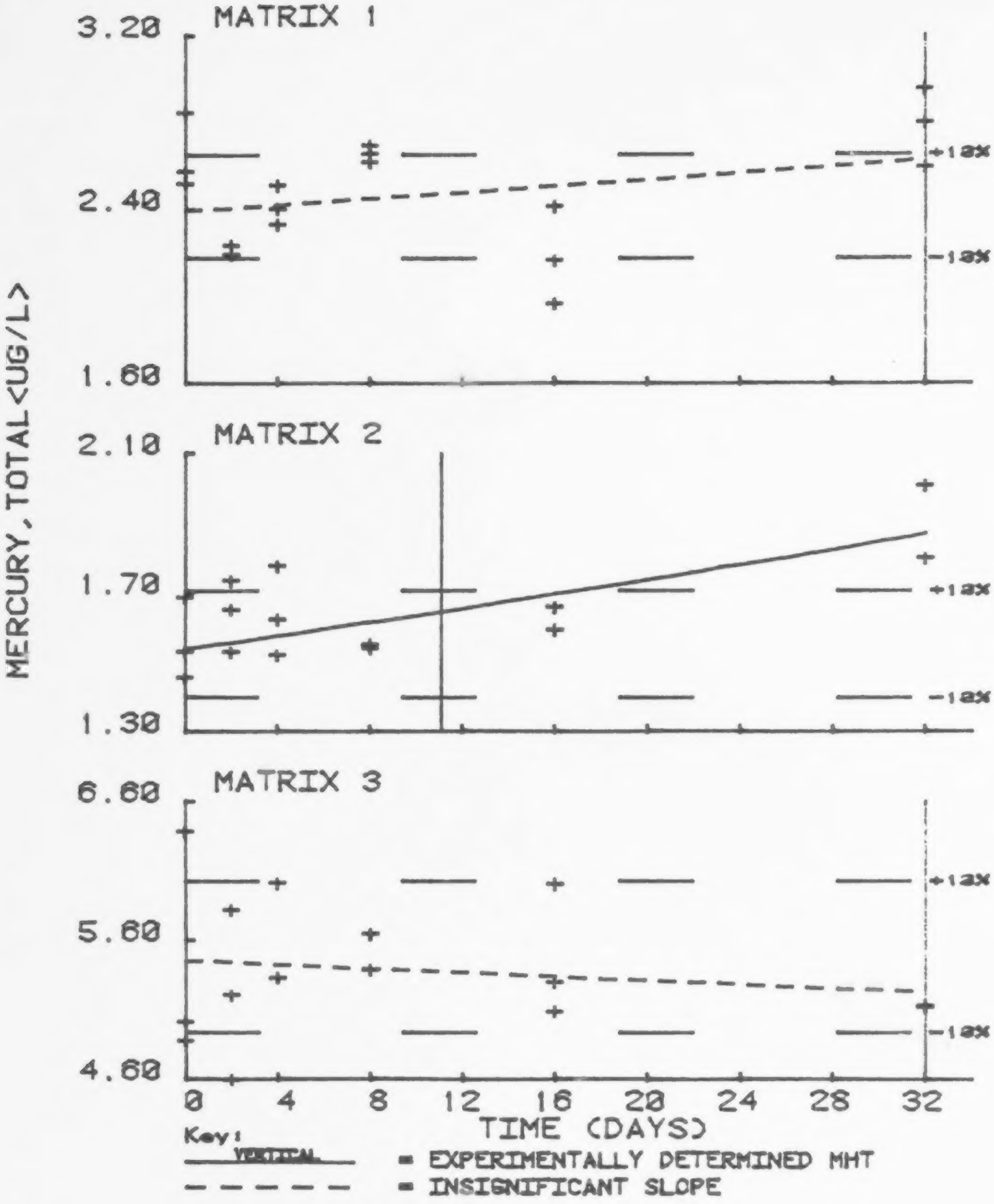


Figure 5-6. STATISTICAL RESULTS OF EXPERIMENT 6 FOR MERCURY

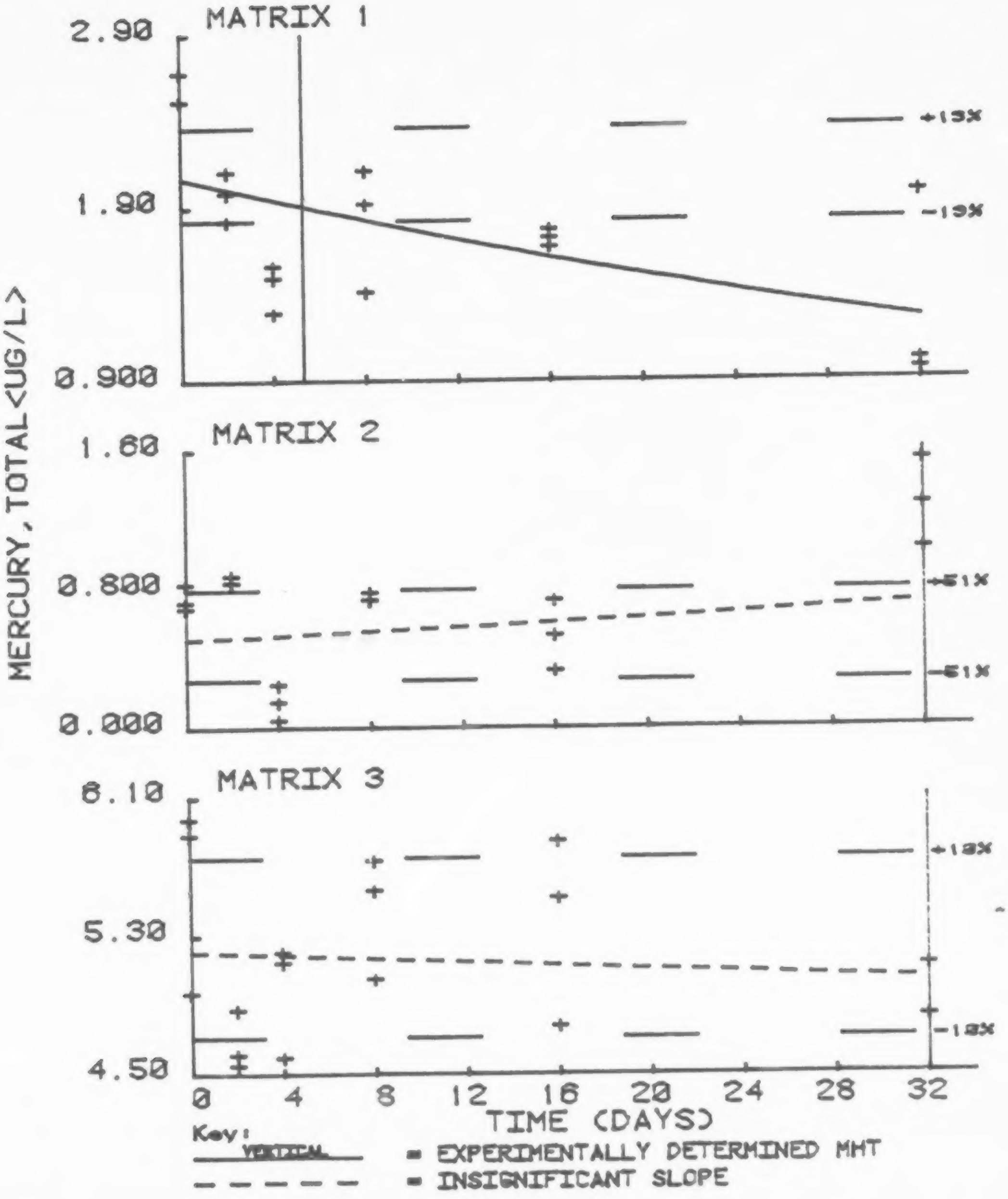


Figure 5-7. STATISTICAL RESULTS OF EXPERIMENT 7 FOR MERCURY

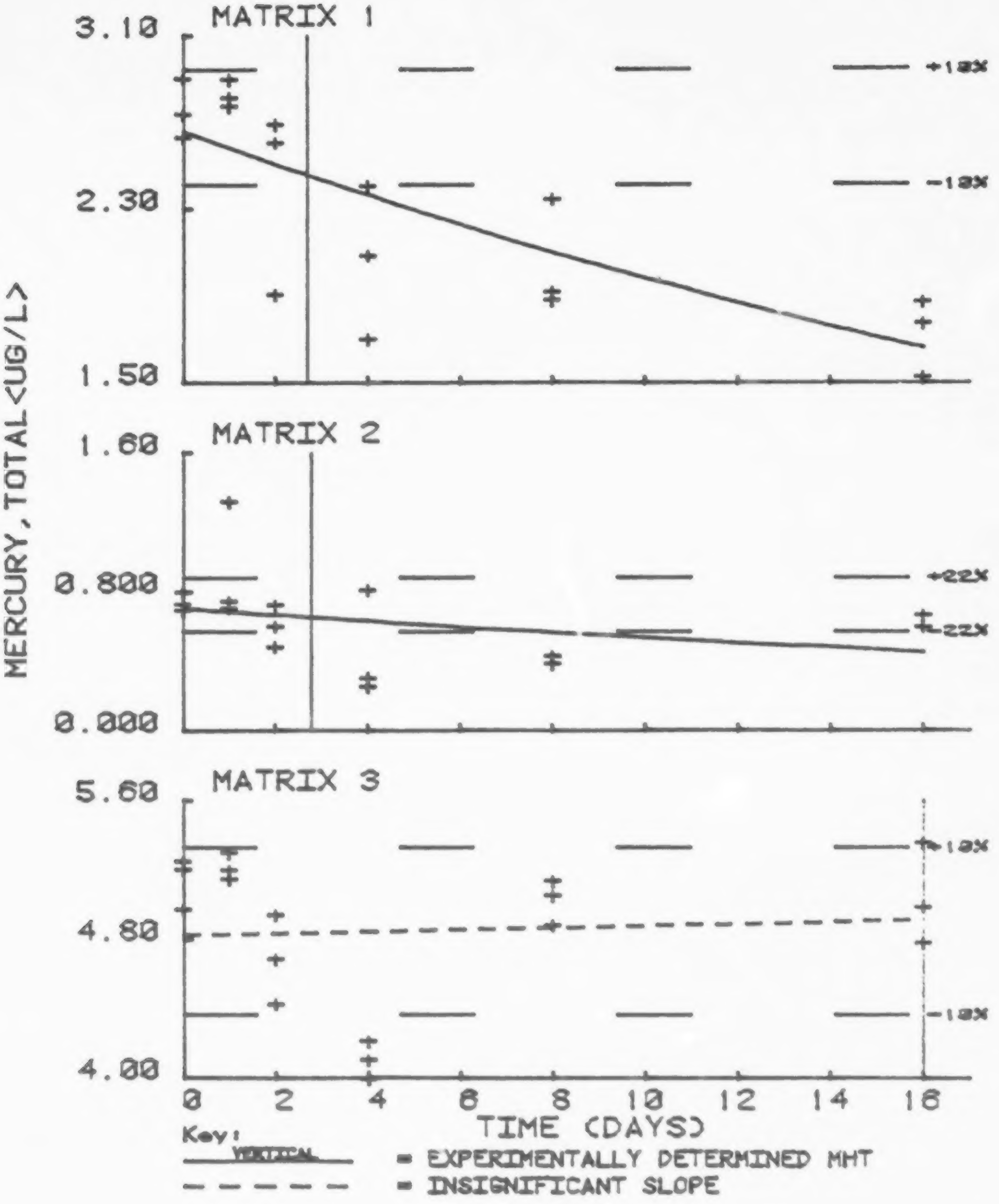


Figure 5-8. STATISTICAL RESULTS OF EXPERIMENT 8 FOR MERCURY

5.4 NITRATE AND NITRATE PLUS NITRITE

5.4.1 Experiments and Results

MHT experiments were performed for two preservation techniques for samples collected for nitrate in drinking water samples and one preservation of nitrate plus nitrite in wastewater samples. Samples for all three experiments were stored in plastic containers. The preservation techniques for the two drinking water experiments (9 and 10) were: (1) pH adjustment with sulfuric acid to a pH of less than 2.0; and (2) storage of samples at a temperature of 4°C. The preservation technique for the wastewater samples (Experiment 15) used H_2SO_4 to adjust the pH to less than 2.0 with cold storage at 4°C.

The automated cadmium reduction method followed by the conventional diazotization-coupling reaction with nitrite was used for both the nitrate and nitrate plus nitrite experiments. The cadmium reduction step was eliminated to determine nitrite in drinking water so nitrate could be calculated from the difference between nitrate plus nitrite and nitrite.

In addition to the possible heterogeneity of the samples dispensed for analyses, sources of variability specific to the method and these experiments were sample dilution to the standard curve range (0 to 0.20 mg-N/L) and neutralization of samples preserved with sulfuric acid prior to analysis.

Samples were collected from three different types of finished waters using different treatment processes. The following three drinking water samples for Experiments 9 and 10 were collected:

Matrix 1--Lime softening of a deep aquifer ground water

Matrix 2--Alum coagulation of a surface water

Matrix 3--Lime softening of a shallow aquifer ground water

Samples for the three wastewater matrices (Experiment 15) were collected for two types of industrial dischargers:

Matrix 1--Nitrogenous Fertilizers (SIC Code 2873)

Matrix 2--Poultry and Eggs (SIC Code 0259)

Matrix 3--Nitrogenous Fertilizers (SIC Code 2873)

Table 5-15 summarizes the data for unpreserved and preserved field samples analyzed during each experiment at time equals Day 0. Since storage at 4°C was the only preservative, preserved field samples for Experiment 10 were not analyzed. No significant differences existed between the means of the unpreserved and preserved field samples for each experiment and matrix. Spiking levels were approximately 1.0, 9.2, and 5.0 mg/L for each matrix, respectively, of Experiments 9 and 10. Spiking levels were 15, 1.0, and 7 mg/L for each matrix, respectively, of Experiment 15.

Tables 5-16, 5-17, and 5-18 present the raw data and summary statistics for nitrate in drinking water (Experiments 9 and 10) and for nitrate plus nitrite in wastewater (Experiment 15), respectively. The CV's reported in these tables of the overall mean nitrate concentrations determined for each time-sequence analysis ranged from 1.43 to 10.79 percent for the three experiments. A better estimate of day-to-day analytical variability for each matrix of the three experiments was determined from the lack-of-linear-fit term of the individual lack-of-fit ANOVA's for the first-order model. The CV's of the lack-fit terms for each matrix were:

Experiment	Matrix	CV (%)
9	1	16.3
	2	7.0
	3	7.5
10	1	6.2
	2	5.6
	3	7.1
15	1	9.6
	2	8.4
	3	1.4

These lack-of-fit variabilities compared favorably with an EPA method study (EPA, 1979a) for three participating laboratories which reported CV ranges of 4 to 26 percent for a concentration range of 0.29 to 2.48 mg-nitrogen (N)/L.

The variability for triplicate samples analyzed at Day 0 was used to evaluate the homogeneity of the dispensed samples. The CV's at Day 0 for each matrix and experiment, which indicated well-mixed samples, were:

Experiment	Matrix	CV (%)
9	1	10.8
	2	3.4
	3	2.8
10	1	2.8
	2	4.4
	3	1.3
15	1	0.6
	2	1.1
	3	0.4

The larger CV for Matrix 1 of Experiment 9 was apparently a result of analyst variability because neutralization of samples was required prior to analysis.

5.4.2 Statistical Evaluation

Generally, the first-order model exhibited the best distributional characteristics (normality and homogeneity) compared to the zero-order model for the standardized residuals of the overall two-way ANOVA's for the three experiments. In most cases, the results from the individual lack-of-fit ANOVA's indicated that the log-concentration-versus-time trends were not best explained by linear fits. Since the variability associated with the lack of linear fit compared favorably with the expected analytical variability, the calculated CT's and LCL's were assumed valid for the first-order model. The precision of the methods used during each time-sequence study was enough to allow a 10 percent concentration change to be significant. The significant (90 percent confidence level) percent changes in concentration from the intercept of the best-fit regression lines were:

Experiment	Matrix	% Change
9	1	5.9
	2	2.4
	3	2.5
10	1	2.6
	2	1.6
	3	1.8
15	1	0.9
	2	2.4
	3	5.2

Figures 5-9 and 5-10 show the statistical results for the nitrate drinking water experiments (9 and 10). The results of the statistical evaluation tests for the drinking water experiments showed that log-concentration-versus-time slopes for all matrices of these experiments were not significantly different than a slope of zero. Therefore, the experimentally determined MHT for all matrices of the two drinking water experiments is 16 days.

Additional samples (shown in Table 5-16) were analyzed for Experiment 15 because the analyses on Day 32 for Matrix 2 were not consistent with previous days results. Figure 5-11 shows the statistical results, including the additional nitrate plus nitrite analyses for Experiment 15 in wastewater, for computation.

The results in days of the initial statistical evaluation tests for the nitrate plus nitrite wastewater experiment (Experiment 15) were:

Experiment	Matrix	CT (days)	LCL (days)
15	1	69	47
	2	22.3	18.4
	3	107	78

The results in days using the additional analyses were:

Experiment	Matrix	CT (days)	LCL (days)
15	1	187	87
	2	88	30.2
	3	107	78

When Day 32 for Matrix 2 is omitted, the calculated CT and LCL were 226 and 53.6 days, respectively. Therefore, the experimentally determined MHT for each matrix of Experiment 15 for nitrate plus nitrite in wastewater is 32 days.

Table 5-15. Nitrate (mg-N/L) and Nitrate Plus Nitrite (mg-N/L) Results for Samples Analyzed at Time = 0.

Nitrate in Drinking Water

MATRIX	EXPERIMENT	SAMPLE TYPE	MEAN (MG-N/L)	STD.DEV. (MG-N/L)
1	9	USUP	0.012	0.001
1	9	USP	0.014	0.003
1	10	USUP	0.021	0.013
2	9	USUP	0.042	0.001
2	9	USP	0.041	0.001
2	10	USUP	0.042	0.001
3	9	USUP	0.043	0.000
3	9	USP	0.048	0.001
3	10	USUP	0.039	0.001

NITRATE PLUS NITRITE IN WASTEWATER

MATRIX	EXPERIMENT	SAMPLE TYPE	MEAN (MG-N/L)	STD.DEV. (MG-N/L)
1	15	USUP	10.59	0.419
1	15	USP	9.85	0.068
2	15	USUP	6.36	0.043
2	15	USP	6.31	0.007
3	15	USUP	39.62	0.122
3	15	USP	39.28	0.201

USUP = UNSPIKED, UNPRESERVED
USP = UNSPIKED, PRESERVED

Table 5-16. Nitrate (mg-N/L) Raw Data and Summary Statistics for Experiment 9.

TIME		MATRIX 1			MATRIX 2			MATRIX 3	
	DATA	MEAN	S.D.	DATA	MEAN	S.D.	DATA	MEAN	S.D.
0	0.969	1.058	0.114	9.536	9.189	0.313	4.868	4.818	0.134
	1.018			9.104			4.665		
	1.186			8.927			4.919		
1	1.099	1.187	0.077	9.493	9.510	0.029	4.984	5.072	0.174
	1.219			9.493			4.959		
	1.243			9.544			5.272		
2	1.282	1.168	0.100	8.710	8.645	0.061	4.513	4.529	0.028
	1.127			8.637			4.561		
	1.096			8.589			4.513		
4	1.173	1.141	0.030	9.209	9.493	0.350	4.558	4.629	0.102
	1.135			9.885			4.583		
	1.113			9.384			4.746		
8	1.451	1.398	0.100	9.488	9.488	0.003	4.939	4.957	0.042
	1.461			9.488			4.926		
	1.283			9.488			5.005		
16	1.133	1.146	0.015	8.687	8.860	0.163	4.926	4.950	0.054
	1.162			8.885			5.012		
	1.143			9.009			4.913		
	MEAN	S.D.	C.V.	MEAN	S.D	C.V.	MEAN	S.D.	C.V.
TOTAL	1.183	0.128	10.79	9.198	0.389	4.226	4.826	0.215	4.465

S.D. = Standard Deviation
C.V. = Coefficient of Variation (percent)
CONC = Concentration in mg-N/L

Table 5-17. Nitrate (mg-n/L) Raw Data and Summary Statistics for Experiment 10.

TIME DATA MATRIX 1 MEAN S.D. DATA MATRIX 2 MEAN S.D. DATA MATRIX 3 MEAN S.D.

TIME		MATRIX 1			MATRIX 2			MATRIX 3	
	DATA	MEAN	S.D.	DATA	MEAN	S.D.	DATA	MEAN	S.D.
0	1.190 1.126 1.171	1.162	0.033	9.155 8.699 8.396	8.750	0.382	4.792 4.716 4.843	4.784	0.064
1	1.263 1.059 1.028	1.117	0.128	8.914 8.939 9.065	8.973	0.081	4.934 4.959 5.034	4.976	0.052
2	1.142 1.067 1.064	1.091	0.044	8.540 8.516 8.637	8.564	0.064	4.464 4.767 4.706	4.646	0.160
4	1.090 1.093 1.070	1.084	0.012	9.560 9.685 8.940	9.395	0.399	4.974 5.133 4.935	5.014	0.105
8	1.068 1.271 1.195	1.178	0.103	8.812 8.613 8.713	8.713	0.100	4.779 4.754 4.978	4.837	0.123
16	1.222 1.123 1.094	1.146	0.067	8.624 8.703 8.938	8.755	0.163	4.816 4.908 4.973	4.899	0.079
	MEAN	S.D.	C.V.	MEAN	S.D	C.V.	MEAN	S.D.	C.V.
TOTAL	1.116	0.069	6.141	8.879	0.324	3.647	4.910	0.198	4.025

S.D. = Standard Deviation
C.V. = Coefficient of Variation (percent)
CONC = Concentration in mg-N/L

Table 5-18. Nitrate Plus Nitrite (mg-N/L) Raw Data
and Summary Statistics for Experiment 15.

TIME	MATRIX 1			MATRIX 2			MATRIX 3		
	DATA	MEAN	S.D.	DATA	MEAN	S.D.	DATA	MEAN	S.D.
0	28.21	28.15	0.157	7.460	7.522	0.079	47.53	47.57	0.184
	28.27			7.495			47.41		
	27.98			7.611			47.77		
2	29.22	28.81	0.442	7.313	7.309	0.018	47.77	47.83	0.112
	28.34			7.289			47.95		
	28.87			7.325			47.77		
4	27.82	27.90	0.329	7.569	7.498	0.179	47.57	47.41	0.140
	28.27			7.294			47.33		
	27.63			7.629			47.33		
8	28.21	28.13	0.093	7.388	7.455	0.059	48.19	48.11	0.267
	28.15			7.482			48.32		
	28.03			7.494			47.81		
16	28.67	28.75	0.139	7.697	7.751	0.094	47.50	47.27	0.321
	28.90			7.697			47.40		
	28.67			7.859			46.90		
32	28.98	29.51	0.461	8.988	8.506	0.424	45.59	46.19	0.620
	29.77			8.341			46.16		
	29.77			8.189			46.83		
57	28.50	28.77	0.253	7.627	7.608	0.034			
	29.00			7.569					
	28.80			7.627					
	MEAN	S.D.	C.V.	MEAN	S.D	C.V.	MEAN	S.D.	C.V.
TOTAL	28.57	0.579	2.028	7.664	0.405	5.282	47.40	0.679	1.432

Blanks in Table Indicate Samples were not Analyzed on that day
S.D. = Standard Deviation
C.V. = Coefficient of Variation (percent)
CONC = Concentration in mg-N/L

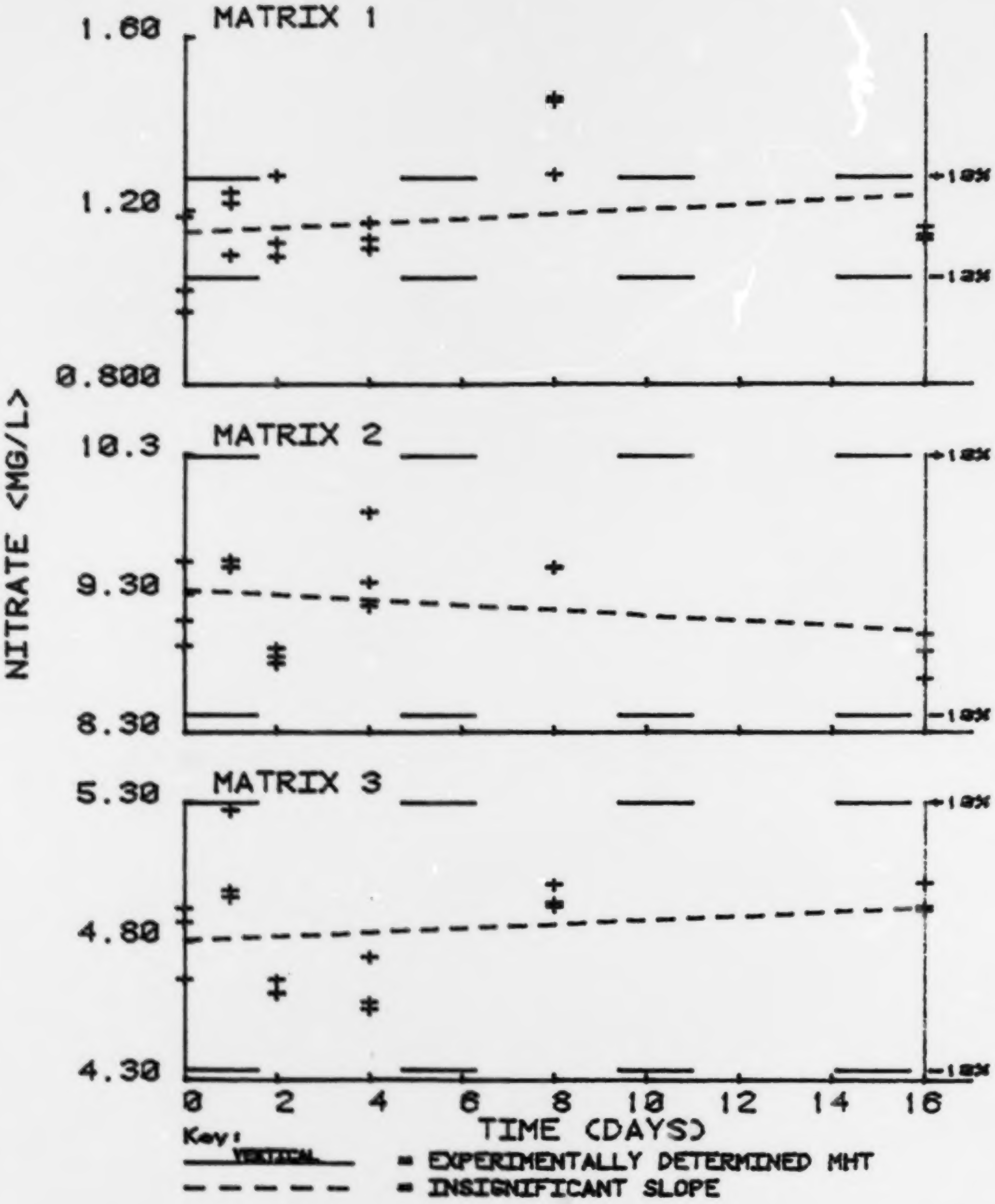


Figure 5-9. STATISTICAL RESULTS OF EXPERIMENT 9 FOR NITRATE

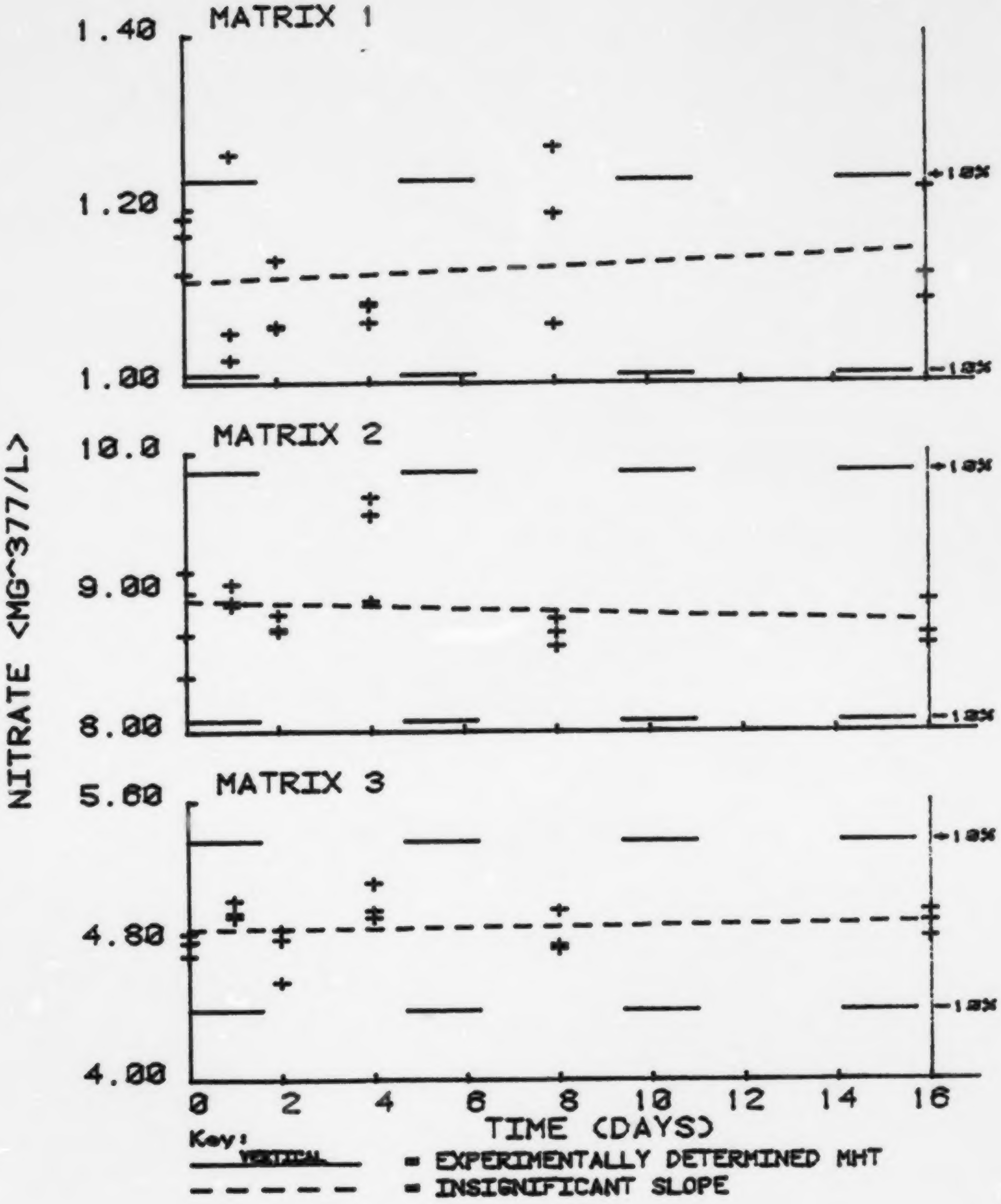


Figure 5-10. STATISTICAL RESULTS OF EXPERIMENT 10 FOR NITRATE

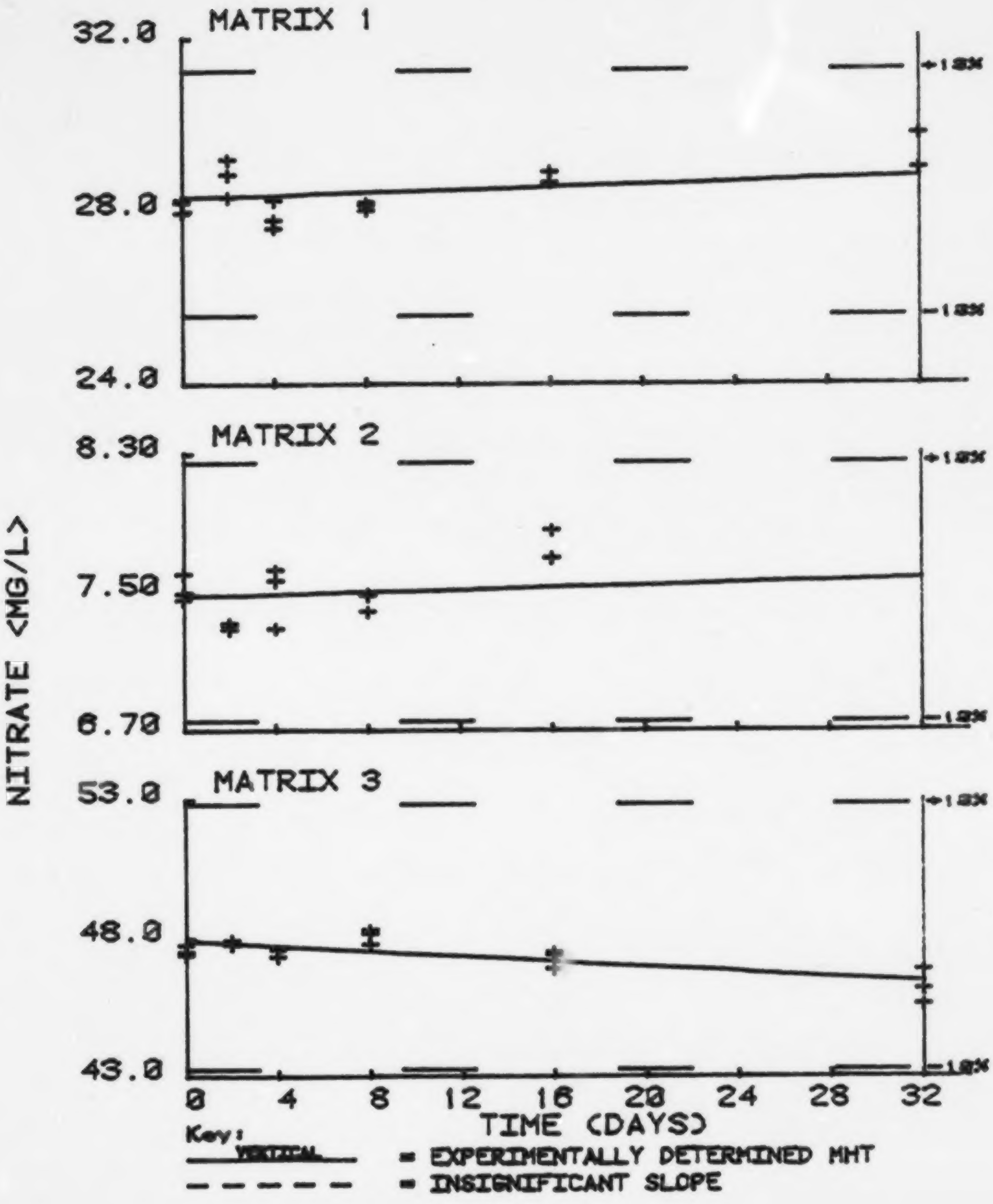


Figure 5-11. STATISTICAL RESULTS OF EXPERIMENT 15 FOR NITRATE

5.5 FLUORIDE

5.5.1 Experiments and Results

Two MHT experiments were performed for fluoride, one in drinking water and one in wastewater samples. Samples for the two experiments were stored in plastic containers with no preservation. The fluoride concentration was determined potentiometrically using the ion-selective electrode for both experiments (EPA Method 340.2); however, the Bellack distillation was used to remove possible interferences prior to the potentiometric measurement of fluoride in the wastewater experiments. Samples containing high levels of fluoride (>10 mg/L) were diluted prior to distillation to prevent loss of fluoride during distillation.

Samples were collected from three different types of finished waters using different treatment processes. The following three drinking water matrices for Experiment 11 were collected:

Matrix 1--Lime softening of a deep aquifer ground water

Matrix 2--Alum coagulation of a surface water

Matrix 3--Lime softening of a shallow aquifer ground water

Samples for the three wastewater matrices (Experiment 13) were collected from three types of industrial dischargers:

Matrix 1--Phosphate Rock (SIC Code 1475)

Matrix 2--Primary Production of Aluminum (SIC Code 3334)

Matrix 3--Primary Smelting and Refining of Nonferrous Metals, NEC
(SIC Code 3339)

Table 5-19 summarizes the data for unpreserved field samples from each experiment at time equals Day 0. Preserved field samples were not analyzed because samples were not preserved. For Experiment 11, the spiking levels were approximately 0.25, 0.25, and 1.0 mg/L for each matrix, respectively. Spiking levels were 1.0, 5.0, and 2.6 mg/L for each matrix, respectively, of Experiment 13.

Tables 5-20 and 5-21 present the raw data and summary statistics for fluoride in drinking water Experiment 11 and in wastewater Experiment 13. The CV's reported in these tables of the overall mean fluoride concentrations determined for each time-sequence study, ranged from 1.63 to 5.43 for the two experiments. A better estimate of day-to-day variability for each matrix of the two experiments was determined from the lack-of-linear-fit term of the individual lack-of-fit ANOVA's for the first-order model (see Section 4.2.7). The CV's from the lack-of-linear-fit terms were:

Experiment	Matrix	CV (%)
11	1	9.5
	2	4.9
	3	3.1
13	1	9.1
	2	1.8
	3	3.0

These lack-of-fit variabilities compared favorably with an EPA method study (1979a) for 111 participating laboratories which reported a CV of 3.5 for a synthetic sample with no interferences (concentration 0.84 mg-flouride (F)/L) and a CV of 4.8 for a synthetic sample with interferences (concentration 0.75 mg-F/L).

The variability for triplicate samples analyzed at Day 0 was used to evaluate the homogeneity of the dispensed samples. The CV's at Day 0 for each matrix and experiment indicating acceptable mixing were:

Experiment	Matrix	CV (%)
11	1	5.8
	2	1.1
	3	0.38
13	1	3.8
	2	2.3
	3	0.40

5.5.2 Statistical Evaluation

The first-order model exhibited the best distributional characteristics (normality and homogeneity) compared to the zero-order model for the standardized residuals of the overall two-way ANOVA's for both experiments. Results from the individual lack-of-fit ANOVA's (for each matrix of both experiments) indicated that the log-concentration-versus-time trends for each matrix were not best explained by linear fits. In other words, day-to-day analytical variability was greater than within-day variability. Since the variability associated with the lack of linear fit compared favorably with expected analytical variability, the calculated CT's and LCL's were assumed valid for the first-order model. The precision of the method allowed a 10 percent change in concentration to be significant. At the 90 percent confidence level, the significant percent changes in concentration from the intercept of the best-fit regression line were:

Experiment	Matrix	% Change
11	1	2.7
	2	1.5
	3	0.8

Experiment	Matrix	% Change
13	1	2.2
	2	0.8
	3	0.9

Additional samples (shown in Tables 5-20 and 5-21) were analyzed for both 32-day experiments following the initial statistical evaluations. The analysis during Day 0 of Matrix 1 for Experiment 11 had fluoride electrode problems. During the analysis of Matrix 1 samples for Experiment 13, unexpected day-to-day variability was encountered due to inaccuracies in determining volumes of the distillates; corrective action involved weighing the distillate for correction of the final concentration. Figures 5-12 and 5-13 show the resulting slopes and experimentally determined MHT's of each matrix, computationally including all additional analyses for the drinking water and wastewater experiments, respectively. The results of the initial statistical evaluation tests for each matrix were:

Experiment	Matrix	CT (days)	LCL (days)
11	1	45.3	12.1
	2	32	32
	3	32	32
13	1	45.9	15.2
	2	120	63.8
	3	32	32

Log-concentration-versus-time slopes for Matrices 2 and 3 of Experiment 11 and Matrix 3 of Experiment 13 were not significantly different than a slope of zero. The results of the statistical evaluation tests using the additional analyses were:

Experiment	Matrix	CT (days)	LCL (days)
11	1	57.6	25.3
	2	32	32
	3	32	32
13	1	32	32
	2	120	63.8
	3	32	32

Using the additional analyses, the slope for Matrix 1 of Experiment 13 was not significantly different than zero. For Matrix 1 of Experiment 11, the calculated CT and LCL were 174 and 53 days, respectively, when results for Day 0 were omitted. Therefore, the experimentally determined MHT for each matrix of Experiments 11 and 13 is 32 days.

Table 5-19. Fluoride (mg/L) Results for Samples Analyzed at Time = 0.

FLUORIDE IN DRINKING WATER

MATRIX	EXPERIMENT	SAMPLE TYPE	MEAN (MG/L)	STD.DEV. (MG/L)
1	11	USUP	0.569	0.018
2	11	USUP	0.123	0.011
3	11	USUP	0.655	0.008

FLUORIDE IN WASTEWATER

MATRIX	EXPERIMENT	SAMPLE TYPE	MEAN (MG/L)	STD.DEV. (MG/L)
1	13	USUP	6.464	0.231
2	13	USUP	28.39	0.417
3	13	USUP	12.76	0.163

USUP = UNSPIKED,UNPRESERVED

Table 5-20. Fluoride (mg/L) Raw Data and Summary Statistics for Experiment 11.

TIME	MATRIX 1			MATRIX 2			MATRIX 3		
	DATA	MEAN	S.D.	DATA	MEAN	S.D.	DATA	MEAN	S.D.
0	0.755	0.808	0.047	0.366	0.370	0.004	1.577	1.583	0.006
	0.824			0.374			1.590		
	0.844			0.371			1.583		
2	0.927	0.919	0.008	0.385	0.374	0.011	1.523	1.535	0.016
	0.916			0.374			1.529		
	0.913			0.362			1.553		
4	0.928	0.924	0.006	0.370	0.370	0.001	1.528	1.534	0.006
	0.928			0.372			1.539		
	0.917			0.369			1.534		
8	0.911	0.909	0.008	0.404	0.399	0.006	1.570	1.601	0.035
	0.915			0.400			1.639		
	0.900			0.392			1.595		
16	0.955	0.955	0.0003	0.393	0.385	0.007	1.563	1.569	0.006
	0.955			0.382			1.575		
	0.955			0.379			1.569		
32	0.931	0.921	0.014	0.379	0.379	0.002	1.565	1.586	0.019
	0.927			0.382			1.590		
	0.905			0.377			1.603		
84	0.967	0.949	0.020	0.412	0.387	0.022	1.581	1.566	0.015
	0.951			0.373			1.568		
	0.928			0.375			1.550		
	MEAN	S.D.	C.V.	MEAN	S.D	C.V.	MEAN	S.D.	C.V.
TOTAL	0.912	0.049	5.425	0.381	0.013	3.436	1.568	0.029	1.819

Blanks in Table Indicate Samples were not Analyzed on that day
S.D. = Standard Deviation
C.V. = Coefficient of Variation (percent)
CONC = Concentration in mg/L

Table 5-21. Fluoride (mg/L) Raw Data and Summary
Statistics for Experiment 13.

TIME		MATRIX 1			MATRIX 2			MATRIX 3	
	DATA	MEAN	S.D.	DATA	MEAN	S.D.	DATA	MEAN	S.D.
0	7.666	7.790	0.296	35.80	34.87	0.811	15.36	15.36	0.062
	8.129			34.33			15.42		
	7.576			34.48			15.30		
2	7.594	7.667	0.266	34.65	34.52	0.498	15.56	15.64	0.094
	7.962			33.96			15.74		
	7.446			34.93			15.62		
4	7.362	7.315	0.108	33.92	34.01	0.155	15.99	15.99	0.126
	7.391			34.19			15.87		
	7.191			33.92			16.12		
8	6.662	6.671	0.016	34.43	33.88	0.547	15.64	15.60	0.255
	6.689			33.34			15.83		
	6.662			33.88			15.32		
16	7.109	7.167	0.058	33.50	33.68	0.205	15.76	15.86	0.096
	7.225			33.90			15.88		
	7.167			33.64			15.94		
23	7.503	7.464	0.044						
	7.415								
	7.473								
32	6.960	7.098	0.139	33.87	33.65	0.277	15.37	15.43	0.061
	7.237			33.34			15.43		
	7.098			33.74			15.49		
100	7.348	7.399	0.046						
	7.409								
	7.439								
	MEAN	S.D.	C.V.	MEAN	S.D	C.V.	MEAN	S.D.	C.V.
TOTAL	7.321	0.360	4.917	34.10	0.610	1.788	15.65	0.255	1.632

Blanks in Table Indicate Samples were not Analyzed on that day
S.D. = Standard Deviation
C.V. = Coefficient of Variation (percent)
CONC = Concentration in mg/L

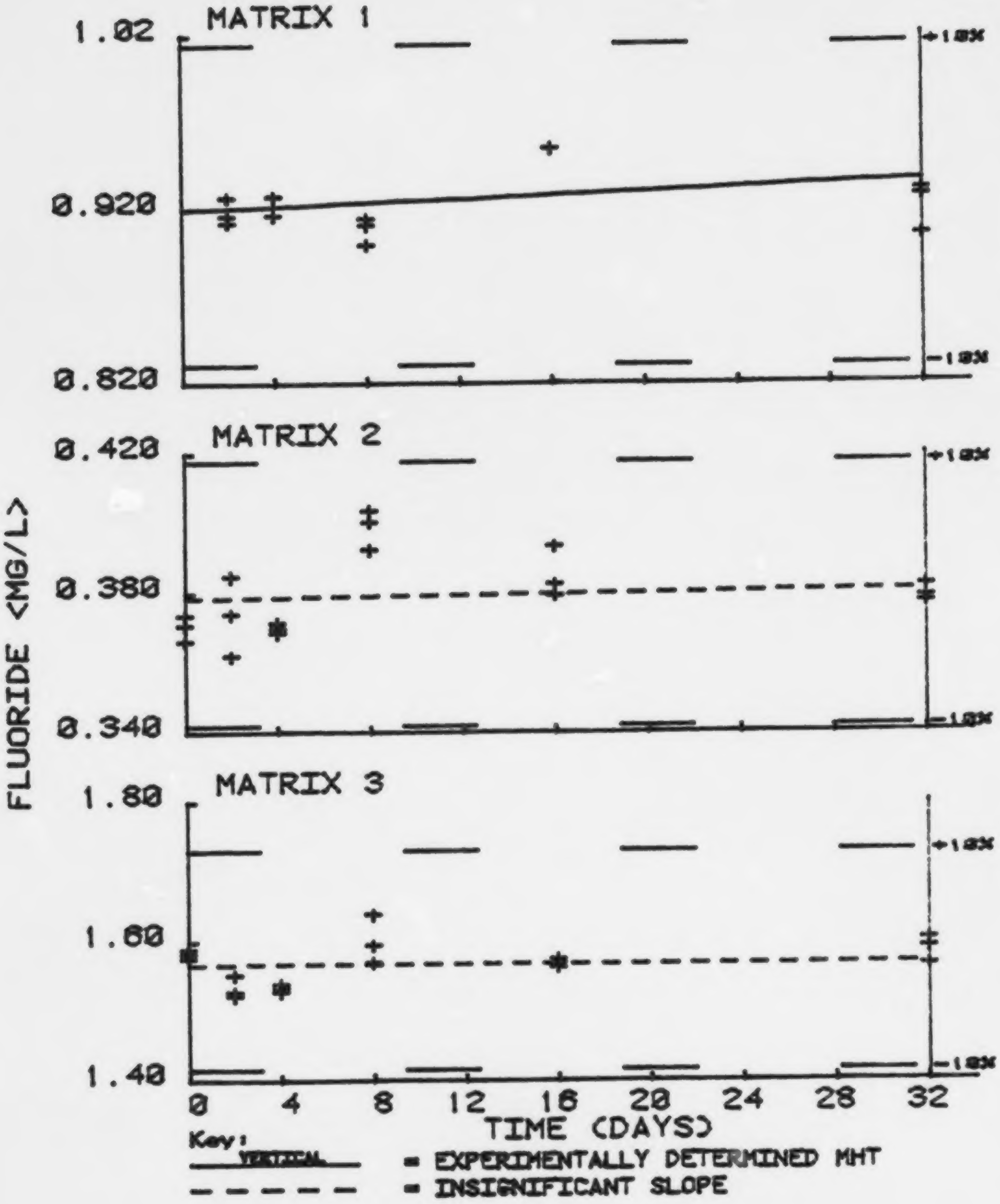


Figure 5-12. STATISTICAL RESULTS OF EXPERIMENT 11 FOR FLUORIDE

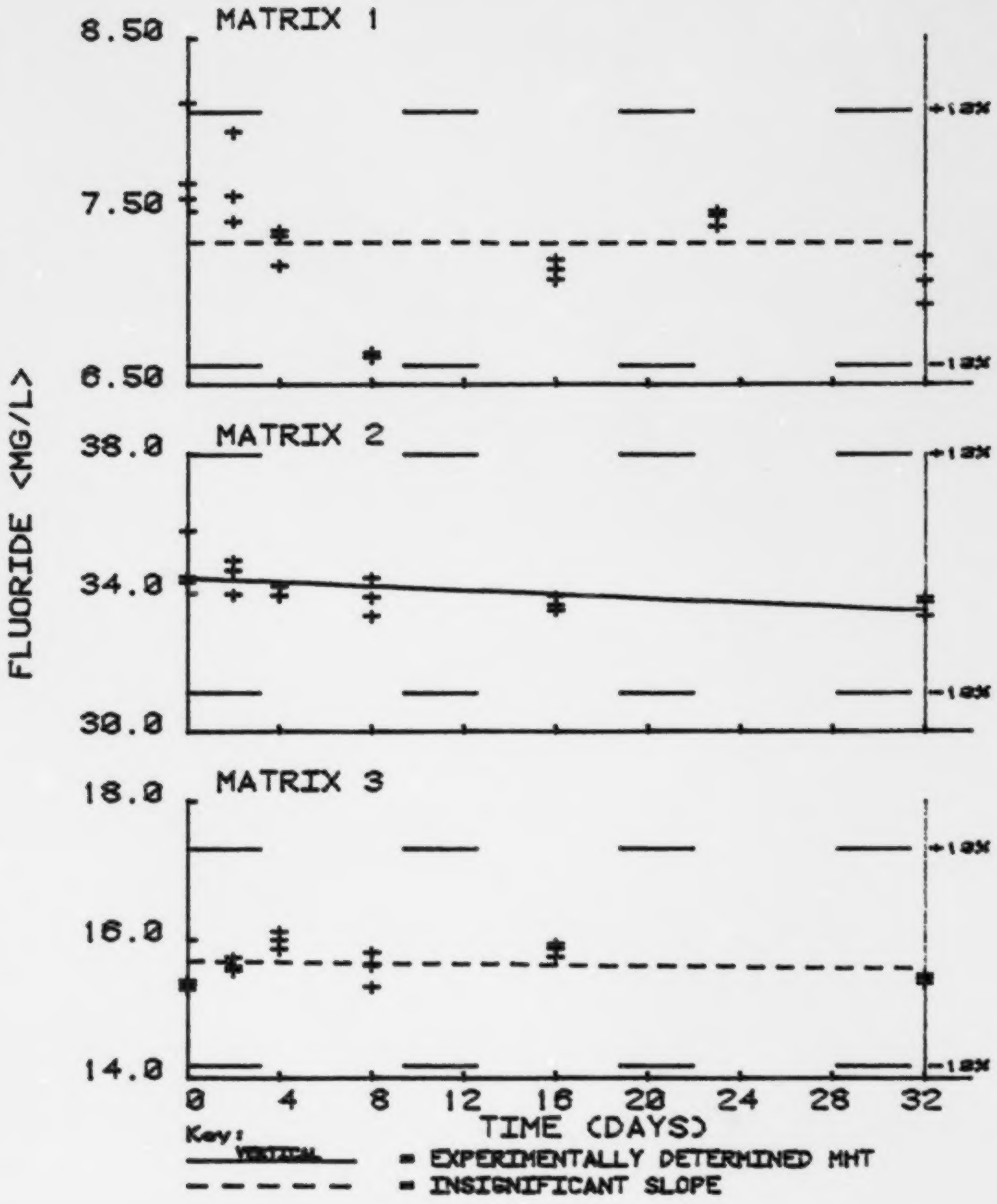


Figure 5-13. STATISTICAL RESULTS OF EXPERIMENT 13 FOR FLUORIDE

5.6 AMMONIA

5.6.1 Experiments and Results

One MHT experiment was conducted for ammonia nitrogen. Samples were stored in plastic containers at 4°C and preserved with H_2SO_4 to a pH of less than 2.0. Distillation followed by analysis of ammonia nitrogen using the colorimetric automated phenate (EPA Method 350.1) was used. Sample sizes used for distillation were adjusted depending on the amount of ammonia nitrogen in the samples.

Samples for three matrices were collected from three types of industrial dischargers:

Matrix 1--Industrial Inorganic Chemicals, NEC (SIC Code 2869);

Matrix 2--Petroleum Refining (SIC Code 2911); and

Matrix 3--Blast Furnaces, Steel Works, and Rolling Mills (SIC Code 3312).

Table 5-22 summarizes the data for unpreserved and preserved field samples analyzed at time equals zero. There were no significant differences between unpreserved and preserved field samples. Spiking levels were approximately 4.2, 1.0, and 0.4 mg/L for each matrix, respectively.

Table 5-23 presents the raw data and summary statistics for the ammonia nitrogen experiment. The CV's reported in this table of the overall mean ammonia concentrations were 4.5, 5.2, and 5.2 percent, respectively. An estimate of day-to-day analytical variability for each matrix was determined from the lack-of-linear-fit term of the individual lack-of-fit ANOVA's for the first-order model. The CV's for the lack-fit terms were 8.0, 10.1, and 7.2 percent for each matrix, respectively.

In a single laboratory, using 1-L surface water samples at concentrations of 1.41, 0.77, 0.59, and 0.43 mg NH_3-N/L, the CV was less than 1 (EPA, 1979a). It is not known whether the variability for the single laboratory was determined on a single day or from several days of analyses or whether or not the samples were distilled. The variability for triplicate samples analyzed at Day 0 was used to evaluate the homogeneity of the dispensed samples used for the time-sequenced studies. The CV at Day 0 for each matrix were 5.5, 2.6, and 0.50 percent, indicating well-mixed samples.

5.6.2 Statistical Evaluation

The first-order model exhibited the best distributional characteristics (normality and homogeneity) compared to the zero-order model for the standardized residuals of the overall two-way ANOVA's. Results from the individual ANOVA's (for each matrix) indicated that the log-concentration-versus-time trends for each matrix were not best explained by linear fits.

In other words, day-to-day analytical variability was greater than within-day variability. The observed lack-of-fit standard deviations are probably a result of normal analytical variability and not due to the need for a higher-order model to explain the data trends. Therefore, the calculated CT's and LCL's are probably valid for the first-order model. The precision of the method during the experiments allowed a 10 percent concentration change to be significant. At the 90 percent confidence level, the significant percent changes in concentration from the intercept of the best-fit regression line were 2.2, 2.4, and 1.8 percent, respectively, for each matrix.

Additional samples were analyzed (shown as Days 58 and 110 on Table 5-23) following the initial statistical evaluation for the 32-day experiment. Figure 5-14 shows the slope results and experimentally determined MHT's using all data only for computations. The initial statistical evaluation test results for each matrix were:

Experiment	Matrix	CT (days)	LCL (days)
12	1	36.2	23.1
	2	45.4	14.5
	3	42.6	27.4

Statistical results using the additional data were:

Experiment	Matrix	CT (days)	LCL (days)
12	1	32	32
	2	32	32
	3	113	77

Therefore, the experimentally determined MHT's for the three matrices of the ammonia experiment are 32 days.

Table 5-22. Ammonia (mg-N/L) Results for Samples Analyzed at Time = 0.

MATRIX	EXPERIMENT	SAMPLE TYPE	MEAN (MG/L)	STD.DEV. (MG/L)
1	12	USUP	5.146	0.031
1	12	USP	5.225	0.013
2	12	USUP	0.813	0.009
2	12	USP	0.801	0.009
3	12	USUP	0.164	0.008
3	12	USP	0.153	0.007

USUP = UNSPIKED,UNPRESERVED
USP = UNSPIKED,PRESERVED

Table 5-23. Ammonia (mg-N/L) Raw Data and Summary Statistics for Experiment 12.

TIME		MATRIX 1			MATRIX 2			MATRIX 3	
	DATA	MEAN	S.D.	DATA	MEAN	S.D.	DATA	MEAN	S.D.
0	9.411 10.11 9.088	9.535	0.520	1.884 1.957 1.890	1.911	0.040	0.606 0.602 0.608	0.606	0.003
2	9.654 9.764 9.819	9.746	0.085	1.776 1.771 1.800	1.782	0.016	0.633 0.627 0.631	0.630	0.003
4	9.049 9.157 9.230	9.145	0.091	1.768 1.763 1.758	1.763	0.005	0.645 0.645 0.647	0.646	0.001
8	9.277 9.167 9.406	9.283	0.120	1.763 1.778 1.763	1.768	0.009	0.577 0.573 0.585	0.578	0.006
16	9.974 9.992 10.01	9.992	0.018	1.554 1.583 1.583	1.573	0.017	0.595 0.589 0.604	0.596	0.007
32	10.24 10.22 10.26	10.24	0.019	1.750 1.750 1.764	1.755	0.009	0.571 0.563 0.577	0.570	0.007
58				1.817 1.817 1.827	1.820	0.006			
110	9.124 9.328 9.217	9.223	0.102	1.725 1.806 1.751	1.761	0.042	0.559 0.574 0.542	0.558	0.016
	MEAN	S.D.	C.V.	MEAN	S.D	C.V.	MEAN	S.D.	C.V.
TOTAL	9.594	0.432	4.500	1.767	0.092	5.181	0.598	0.031	5.178

Blanks in Table Indicate Samples were not Analyzed on that day
S.D. = Standard Deviation
C.V. = Coefficient of Variation (percent)
CONC = Concentration in mg-N/L

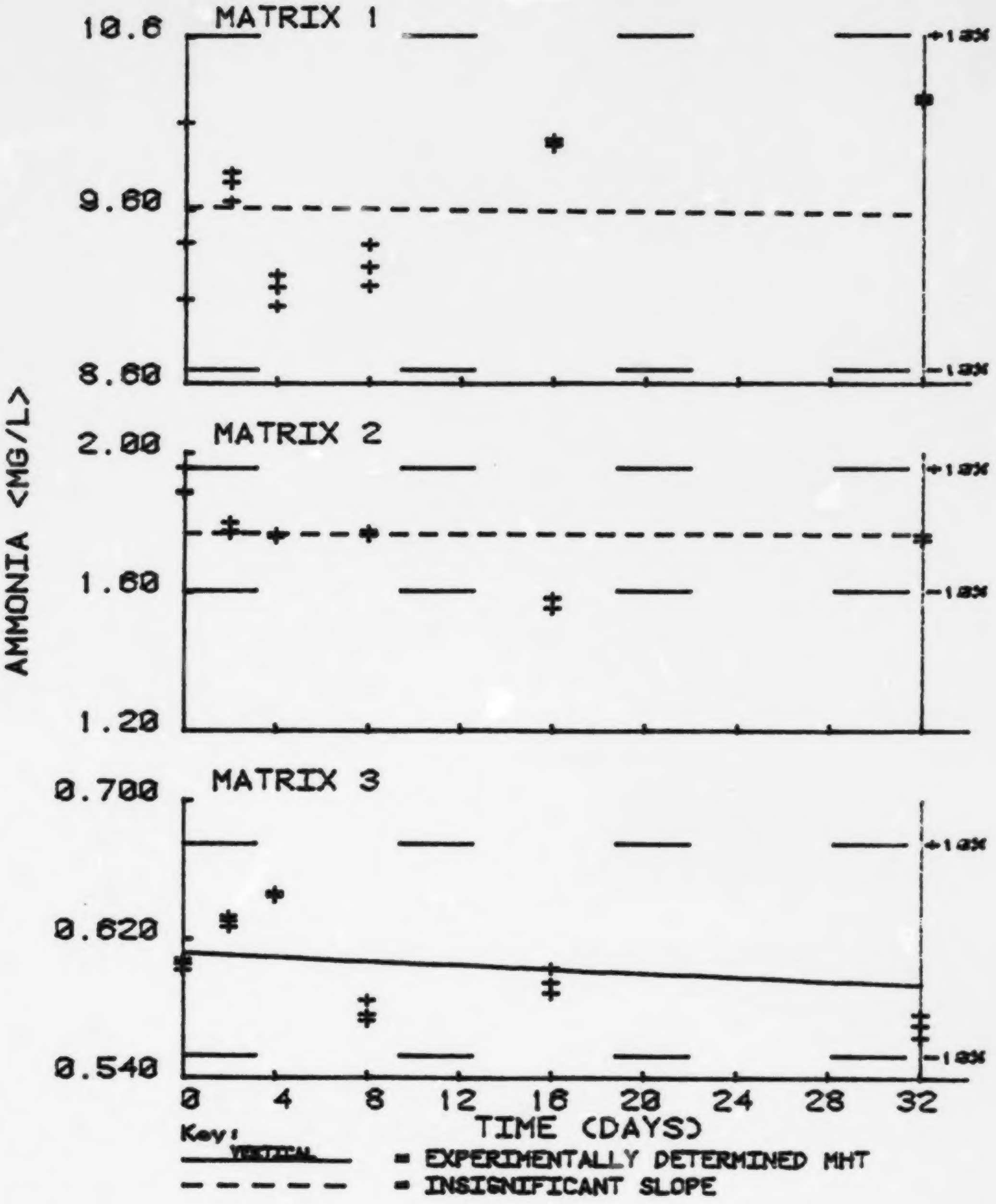


Figure 5-14. STATISTICAL RESULTS OF EXPERIMENT 12 FOR AMMONIA

5.7 TOTAL KJELDAHL NITROGEN

5.7.1 Experiments and Results

One experiment was conducted for TKN. The samples were stored in plastic containers at 4°C and preserved with sulfuric acid to a pH less than 2. Macro-Kjeldahl digestion and distillation followed by analysis using the colorimetric automated phenate method (EPA Method 331.3) was used. Sample sizes used for digestion and distillation were determined by the amount of Kjeldahl nitrogen in the samples.

Samples for three matrices were collected from three types of industrial dischargers:

Matrix 1--Nitrogenous Fertilizers (SIC Code 2873),

Matrix 2--Poultry and Eggs (SIC Code 0259), and

Matrix 3--Industrial Organic Compounds (SIC Code 2869).

Table 5-24 summarizes the data for unpreserved and preserved field samples analyzed at time equals zero. There were no significant concentration differences between the unpreserved and preserved samples. Spiking levels were approximately 25, 1.0, and 1.0 mg/L for each matrix, respectively.

Table 5-25 presents the raw data and summary for the TKN experiment. The CV's of the concentrations observed for the combined times for each matrix were 4.1, 7.3, and 6.1 percent, respectively. An estimate of day-to-day analytical variability for each matrix was determined from the lack-fit term of the individual lack-of-fit ANOVA's or the first-order model analysis of variances. The CV's from the lack-of-linear-fit terms were 7.7, 10.9, and 9.8 percent for each matrix, respectively. These observed lack-of-fit variabilities compare favorably with results of the FWPCA Method Study 2, Nutrient Analyses (EPA, 1979a) using the macro-Kjeldahl method and either titrimetric, colorimetric, or potentiometric analysis. Thirty-one analysts from 20 laboratories analyzed natural water samples at concentrations of 0.20 to 4.61 mg N/L with reported CV of 98.5 to 25.8 percent.

The CV's at Day 0 for each matrix were 4.1, 6.3, and 0.35 percent, indicating well-mixed samples. The slightly large CV for Matrix 2 indicated that a slight heterogeneity situation existed. Subsequent analysis of selected samples indicated heterogeneity of samples greater than 80 in the numerical sequence of dispensed samples. However, statistical evaluation of Matrix 2 indicated that the heterogeneity was small enough not to affect the MHT.

5.7.2 Statistical Evaluation

The first-order model exhibited the best distributional characteristics (normality and homogeneity) compared to the zero-order model for the standardized residuals of the overall two-way ANOVA. Results from the individual lack-of-fit ANOVA's (for each matrix) indicated that the log-concentration-versus-time trends for each matrix were not best explained by linear fits. Since the variability associated with the lack of linear fit compared favorably to the expected analytical variability, the calculated CT's and LCL's were assumed valid. The precision of the method used allowed a 10 percent concentration to be significant. At the 90 percent confidence level, significant changes in concentration from the intercept of the best-fit regression line were 1.9, 3.5, and 2.3 percent for each matrix, respectively.

Additional samples were analyzed (shown on Table 5-25 as Day 134) following the initial statistical evaluation for the 32-day experiment. Figure 5-15 shows the slope results and experimentally determined MHT's using all data for computations. The initial statistical evaluation test results for each matrix were:

Experiment	Matrix	CT (days)	LCL (days)
14	1	41.1	22.8
	2	32	32
	3	28.3	19.0

The slope test for Matrix 2 was not significantly different than a slope of zero. Statistical results using the additional data were:

CT's--32, 32, and 110 days

LCL's--32, 32, and 65 days.

Therefore, the experimentally determined MHT's for the three matrices of the TKN experiment are 32 days.

Table 5-24. TKN (mg-N/L) Results for Samples Analyzed at Time = 0.

MATRIX	EXPERIMENT	SAMPLE TYPE	MEAN (MG/L)	STD.DEV. (MG/L)
1	14	USUP	106.7	1.21
1	14	USP	104.4	2.26
2	14	USUP	5.33	0.045
2	14	USP	5.38	0.119
3	14	USUP	0.485	0.019
3	14	USP	0.523	0.015

USUP = UNSPIKED,UNPRESERVED
USP = UNSPIKED,PRESERVED

Table 5-25. TKN (mg-N/L) Raw Data and Summary Statistics for Experiment 14.

TIME	MATRIX 1			MATRIX 2			MATRIX 3		
	DATA	MEAN	S.D.	DATA	MEAN	S.D.	DATA	MEAN	S.D.
0	134.8	131.3	5.321	6.308	6.021	0.375	1.434	1.429	0.005
	125.2			6.159			1.429		
	134.0			5.597			1.424		
2	125.4	127.8	2.034	5.615	6.334	0.623	1.558	1.570	0.010
	129.1			6.694			1.577		
	128.8			6.694			1.574		
4	123.6	123.2	0.482	5.605	6.328	0.627	1.548	1.553	0.004
	123.3			6.684			1.555		
	122.6			6.696			1.555		
8	132.9	134.6	1.505	7.054	7.050	0.009	1.416	1.412	0.008
	135.5			7.043			1.404		
	135.5			7.054			1.418		
16	137.9	138.2	0.428	6.815	6.979	0.192	1.451	1.467	0.013
	137.9			7.190			1.473		
	138.7			6.933			1.475		
32	136.7	136.3	0.703	6.404	6.425	0.047	1.315	1.330	0.027
	136.7			6.392			1.361		
	135.5			6.479			1.313		
134	127.9	128.4	1.540	6.208	6.406	0.194	1.329	1.352	0.024
	130.1			6.597			1.351		
	127.1			6.412			1.377		
	MEAN	S.D.	C.V.	MEAN	S.D	C.V.	MEAN	S.D.	C.V.
TOTAL	131.4	5.424	4.128	6.506	0.474	7.286	1.445	0.088	6.117

S.D. = Standard Deviation
C.V. = Coefficient of Variation (percent)
CONC = Concentration in mg-N/L

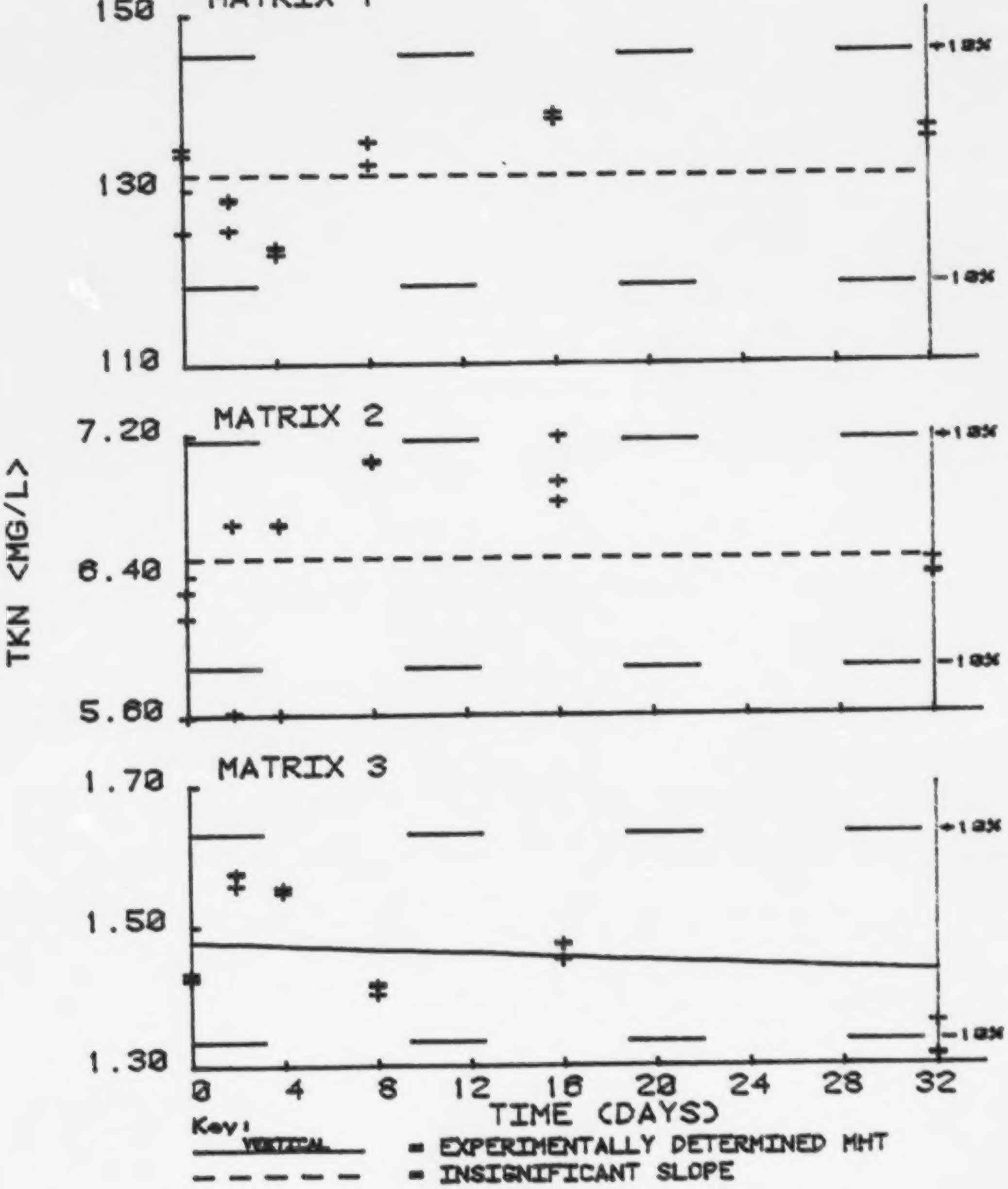


Figure 5-15. STATISTICAL RESULTS OF EXPERIMENT 14 FOR TKN

5.8 TOTAL ORGANIC CARBON

5.8.1 Experiments and Results

One MHT experiment was conducted for total organic carbon (TOC). Samples were stored in plastic containers at 4°C and preserved with sulfuric acid to a pH less than 2.0. Analyses were performed using wet oxidation with potassium persulfate in sealed ampules. The generated carbon dioxide (CO_2) was then purged into an infrared detector for measurement (EPA Method 415.1). An OI-TOC Analyzer was used. Sample size used for analysis varied depending on the concentration range expected.

Samples for three matrices were collected from three types of industrial dischargers:

Matrix 1--Petroleum Refining (SIC Code 2911)

Matrix 2--Industrial Organic Chemicals, NEC (SIC Code 2869)

Matrix 3--Pesticides and Agricultural Chemicals, NEC (SIC Code 2879)

Table 5-26 summarizes the data for unpreserved and preserved field samples analyzed at time equals zero. There were no significant differences between unpreserved and preserved field samples for Matrices 2 and 3. The significant difference for Matrix 1 was due to an apparent outlier for one of the three unpreserved analyses. Spiking levels were approximately 35, 5, and 15 mg/L, respectively.

Table 5-27 presents the raw data and summary statistics for the TOC experiment. The CV's of the concentrations observed for the combined times for each matrix were 4.60, 5.70, and 5.28 percent, respectively. An estimate of day-to-day analytical variability for each matrix was determined from the lack-fit term of the individual lack of fit ANOVA for the first-order model. The CV's from the lack-fit terms were 7.4, 9.1, and 9.2 percent for each matrix, respectively. These observed lack-of-fit variabilities compare favorably with the FWPCA Method Study 3, Demand Analyses (EPA, 1979a) which reported for an interlaboratory study CV's values of 80.2 and 7.78 percent for concentrations of 4.9 and 107 mg-carbon (C)/L. The CV's at Day 0 for each matrix were 2.9, 2.2, and 4.6 percent, indicating well-mixed samples.

5.8.2 Statistical Evaluation

The first-order model exhibited the best distributional characteristics (normality and homogeneity) compared to the zero-order model for the standardized residuals of the overall two-way ANOVA's. Results from the individual lack-of-fit ANOVA's (for each matrix) indicated that the log-concentration-versus-time trends for each matrix were not best explained by linear fits. Since the variability associated with the lack of linear fit compared favorably to expected analytical variability, the results from the lack-of-fit ANOVA's could be overridden, and the calculated CT's and

LCL's were assumed valid. The precision of the method allowed a 10 percent concentration change to be significant. At the 90 percent confidence level, significant percent changes in concentration from the intercept of the best-fit regression line were 2.6, 3.3, and 3.2 percent for each matrix, respectively.

Figure 5-16 shows the results for the first-order model. Slope test results for each matrix indicated that the slopes for all three matrices were not significantly different than a slope of zero (no change). Therefore, the experimentally determined MHT's for the three matrices of the TOC experiment are the maximum time of the experiment (32 days).

Table 5-26. Total Organic Carbon (mg/L) Results for Samples Analyzed at Time = 0.

MATRIX	EXPERIMENT	SAMPLE TYPE	MEAN (MG/L)	STD.DEV. (MG/L)
1	16	USUP	21.248	6.896
1	16	USP	13.594	0.710
2	16	USUP	2.795	0.282
2	16	USP	3.102	0.225
3	16	USUP	14.761	0.748
3	16	USP	13.993	0.750

USUP = UNSPIKED,UNPRESERVED
USP = UNSPIKED,PRESERVED

Table 5-27. TOC (mg/l) Raw Data and Summary Statistics for Experiment 16.

TIME TIME	DATA	MATRIX 1 MATRIX 1 MEAN	S.D.	DATA	MATRIX 2 MATRIX 2 MEAN	S.D.	DATA	MATRIX 3 MATRIX 3 MEAN	S.D.
0	59.59 62.85 60.06	60.83	1.761	6.868 6.744 7.044	6.885	0.151	31.30 28.83 29.10	31.30	
2	55.83 52.16 56.63	54.87	2.384	6.777 6.516 6.520	6.604	0.149	29.02 30.02 28.05	29.02	
4	58.37 54.59 53.50	55.49	2.555	7.264 6.916 7.119	7.100	0.175	28.97 29.75 30.51	28.97	
8	57.43 57.34 57.45	57.40	0.063	6.818 7.078 7.605	7.167	0.401	27.07 26.68 27.76	27.07	
16	57.31 56.39 56.28	56.66	0.566	7.566 7.686 7.178	7.477	0.265	28.07 27.54 26.76	28.07	
32	57.55 58.36 61.80	59.24	2.255	6.921 6.124 6.937	6.661	0.465	29.91 31.54 30.96	30.80	0.827
50	55.71 57.90 57.83	57.15	1.241	7.517 7.480	7.499	0.026			
91	66.26 67.18 68.86	67.43	1.318						
	MEAN	S.D.	C.V.	MEAN	S.D	C.V.	MEAN	S.D.	C.V.
TOTAL	58.63	4.123	7.031	7.034	0.409	5.808	28.99	1.532	5.283

S.D. = Standard Deviation
C.V. = Coefficient of Variation (percent)
CONC = Concentration in mg/L

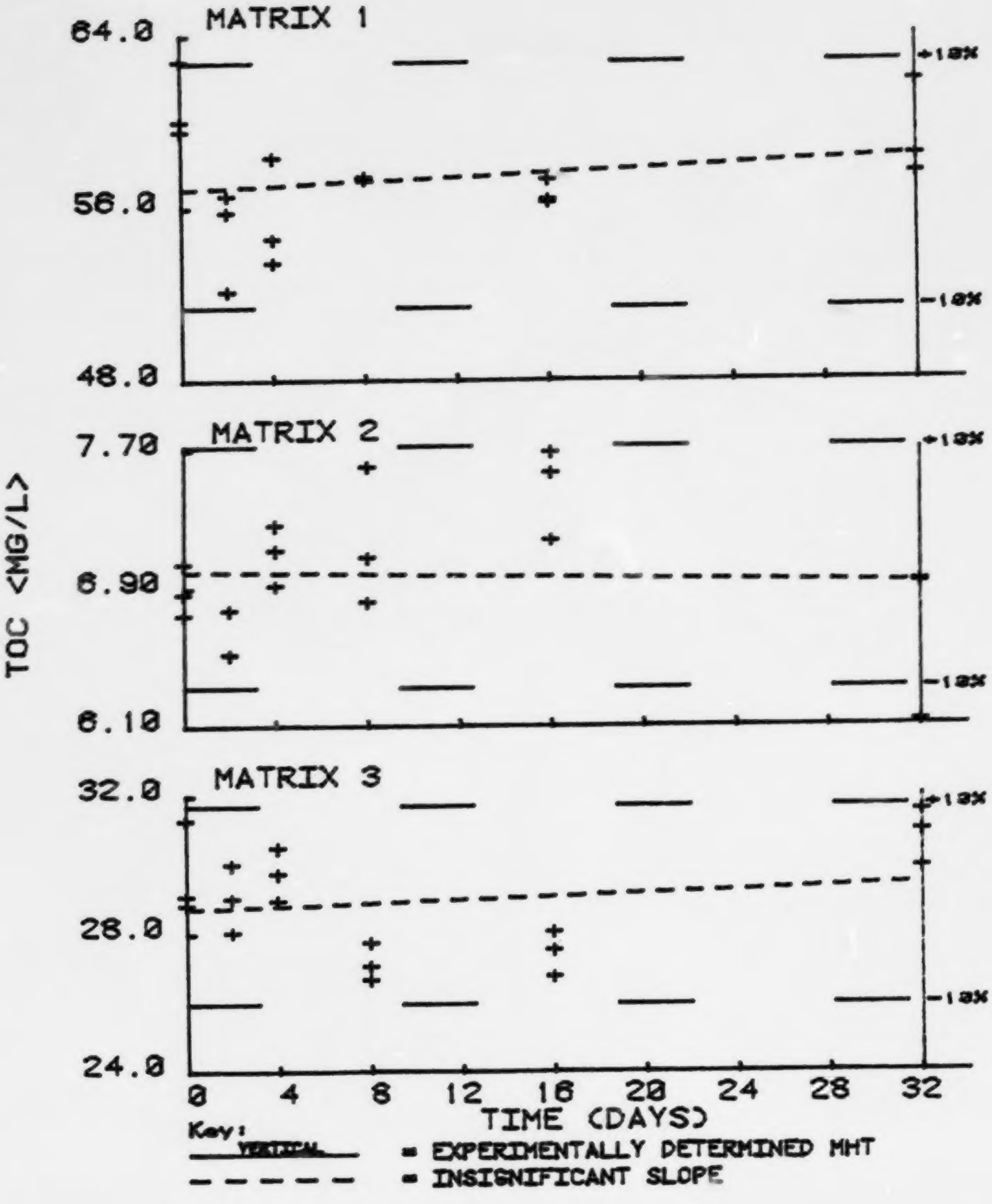


Figure 5-16. STATISTICAL RESULTS OF EXPERIMENT 16 FOR TOC

5.9 TOTAL PHOSPHORUS

5.9.1 Experiments and Results

One MHT experiment was conducted for total phosphorus. Samples were stored in plastic containers at 4°C and preserved with sulfuric acid to a pH of less than 2.0. The persulfate digestion followed by colorimetry was used for analysis of total phosphorus samples. In addition to the possible heterogeneity of the samples dispensed for analyses, a source of variability specific to the method and sample matrices was sample dilution to the working standard curve range of 0 to 0.20 mg-phosphorus (P)/L.

Samples for three matrices were collected from the following three types of industrial dischargers:

Matrix 1--Phosphate Rock (SIC Code 1475)

Matrix 2--Electrical Services (SIC Code 4911)

Matrix 3--Steel Works (SIC Code 3312)

Table 5-28 summarizes the data for unpreserved and preserved field samples analyzed at time equals zero. There were no significant differences between these samples. Spiking levels for the time-sequence studies were approximately 1.0, 0.15, and 0.55 mg/L for each matrix, respectively.

Table 5-29 presents the raw data and summary statistics for the total phosphorus experiment. The CV's reported in this table of the overall mean total phosphorus determined for each time-sequenced study were 0.90, 4.19, and 2.24 percent, respectively. An estimate of analytical variability for each matrix was determined from the lack of linear fit term of the individual lack-of-fit ANOVA's for the first-order model. The CV's from the lack-fit terms were 1.4, 7.6, and 3.6 percent for each matrix, respectively. These observed lack-of-fit variabilities compare favorably with analytical variability reported by an EPA study (1979a) for six participating laboratories which reported CV ranges of 48 to 22 percent for a concentration range of 0.04 to 0.30 mg-P/L. In a single EPA laboratory using surface water samples ranging from 0.04 to 0.84 mg-P/L, the CV ranged from 12.5 to 0 percent. It is not known whether the variability for the single laboratory was determined on a single day or from several days of analyses. The CV's at Day 0 for each matrix were 0.3, 0.4, and 2.5 percent, indicating well-mixed samples.

5.9.2 Statistical Evaluation

The first-order model exhibited the best distributional characteristics (normality and homogeneity) compared to the zero-order model for the standardized residuals of the overall two-way ANOVA's. Results from the individual lack-of-fit ANOVA's (for each matrix) indicated that the log-concentration-versus-time trends for each matrix were not best explained by linear fits. Since the variability associated with the lack of linear fit compared favorably to the expected analytical variability, the results of the lack-of-fit ANOVA's could be overridden and the calculated CT's and LCL's considered valid for the first-order model. The precision of the method allowed a 10 percent concentration change to be significant. At the 90 percent confidence level, significant percent changes in concentrations for the intercept of the best-fit regression line were 0.5, 2.3, and 1.2 percent for each matrix, respectively.

Figure 5-17 shows the results for the first-order model. Slope test results indicated that the slope for Matrices 1 and 2 were not significantly different than a slope of zero (no change in concentration). Therefore, the calculated CT and LCL were the maximum time of the experiment (32 days). The slope for Matrix 3 was significantly different than zero. The calculated CT for Matrix 3 was 95 days with an LCL of 38 days.

Therefore, the experimentally determined MHT's for each matrix of the total phosphorus experiment are 32 days.

Table 5-28. Total Phosphorus (mg-P/L) Results for Samples Analyzed at Time = 0.

MATRIX	EXPERIMENT	SAMPLE TYPE	MEAN (MG-P/L)	STD.DEV. (MG-P/L)
1	17	USUP	9.191	0.031
1	17	USP	9.226	0.015
2	17	USUP	0.136	0.009
2	17	USP	0.118	0.001
3	17	USUP	0.047	0.001
3	17	USP	0.044	0.002

USUP = UNSPIKED,UNPRESERVED
USP = UNSPIKED,PRESERVED

Table 5-29. Total Phosphorus (mg/L) Raw Data and Summary Statistics for Experiment 17.

TIME		MATRIX 1			MATRIX 2			MATRIX 3	
	DATA	MEAN	S.D.	DATA	MEAN	S.D.	DATA	MEAN	S.D.
0	10.17	10.18	0.027	0.265	0.264	0.001	0.572	0.589	0.015
	10.17			0.262			0.600		
	10.21			0.265			0.593		
2	10.07	10.13	0.101	0.296	0.288	0.007	0.612	0.618	0.005
	10.24			0.282			0.622		
	10.07			0.285			0.619		
4	10.44	10.31	0.118	0.256	0.259	0.003	0.623	0.617	0.005
	10.29			0.262			0.614		
	10.20			0.257			0.614		
8	10.20	10.23	0.030	0.281	0.283	0.002	0.619	0.622	0.003
	10.25			0.282			0.625		
	10.22			0.285			0.621		
16	10.29	10.32	0.030	0.276	0.280	0.004	0.620	0.623	0.005
	10.32			0.280			0.621		
	10.35			0.285			0.628		
32	10.17	10.19	0.035	0.278	0.282	0.004	0.620	0.622	0.002
	10.17			0.285			0.624		
	10.23			0.285			0.623		
	MEAN	S.D.	C.V.	MEAN	S.D	C.V.	MEAN	S.D.	C.V.
TOTAL	10.23	0.092	0.901	0.276	0.012	4.194	0.615	0.014	2.542

S.D. = Standard Deviation
C.V. = Coefficient of Variation (percent)
CONC = Concentration in mg-P/L

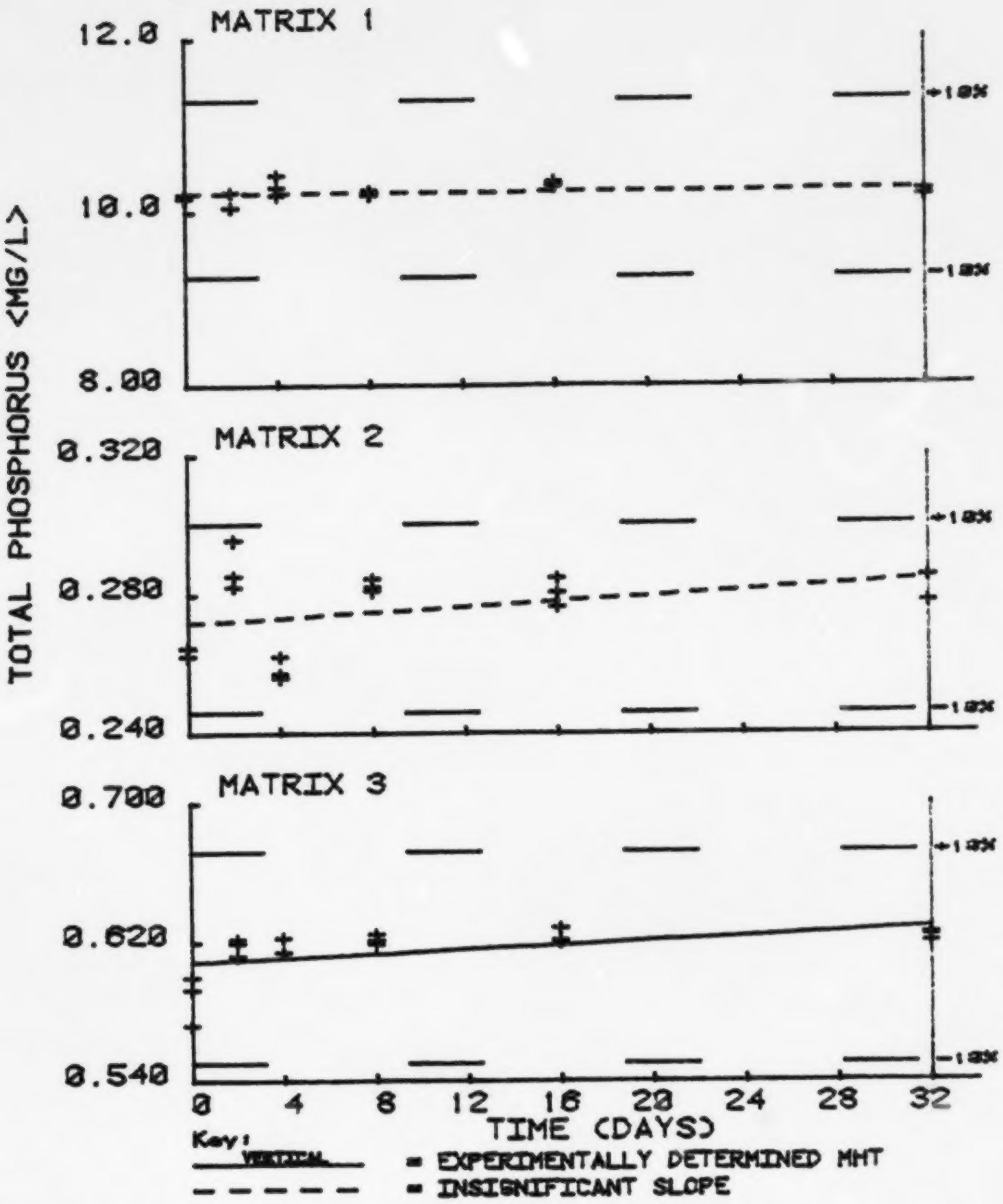


Figure 5-17. STATISTICAL RESULTS OF EXPERIMENT 17 FOR PHOSPHORUS

5.10 SULFIDE

5.10.1 Experiments And Results

One MHT experiment was conducted for sulfide. Samples were stored in plastic containers at 4°C and preserved with 2 mL/L of 2N zinc acetate. The methylene blue colorimetric method (EPA Method 376.2) was used for analysis of sulfide samples. Care was taken by the analyst to remove dissolved oxygen by purging the deionized water with nitrogen gas prior to use for standards.

Samples for three matrices were collected from the following three types of industrial dischargers:

1. Petroleum Refining (SIC Code 2911)

2. Broad Woven Fabric Mills, Cotton (SIC Code 2211)

3. Leather Tanning and Finishing (SIC Code 3111)

Table 5-30 summarizes the data for unpreserved and preserved field samples analyzed at time equals zero. These analyses, at time equals zero, were performed within 48 hours after sample collection. Preservatives (other than chilling at 4°C) were added within 24 to 30 hours after sample collection during preparation of sample aliquots for holding time studies. Preserved sample concentrations were larger than unpreserved sample concentrations because of the time interval between preservation and analysis. Spiking levels were approximately 0.55, 0.10, and 0.75 mg/L for each matrix, respectively.

Table 5-31 presents the raw data and summary statistics for the sulfide experiment. The CV's reported in this table of the overall mean sulfide concentrations determined for each time-sequenced study were 8.0, 10.8, and 6.2 percent, respectively. An estimate of day-to-day analytical variability for each matrix was determined from the lack-of-linear-fit term of the individual lack-of-fit ANOVA's. The CV's from the lack-of-linear-fit terms were 11.9, 18.9, and 9.6 percent for each matrix, respectively. Analytical variability has not been determined for EPA Method 376.2, but the accuracy is approximately plus or minus 10 percent (EPA, 1979; APHA, 1980). The CV's at Day 0 for each matrix were 3.6, 3.9, and 0.39 percent, indicating well-mixed samples.

5.10.2 Statistical Evaluation

The first-order model exhibited the best distributional characteristics (normality and homogeneity) compared to the zero-order model for the standardized residuals of the overall two-way ANOVA's. Results from the individual lack-of-fit ANOVA's for each matrix indicated that the log-concentration-versus-time trends for each matrix were not best explained by linear fits. The observed lack-of-fit standard deviations are probably a result of normal analytical variability and not due to the need for a higher-order model to explain the data trends. Therefore, the calculated CT's and LCL's are probably valid for the first-order model. The precision of the method allowed a 10 percent concentration change to be significant. At the 90 percent confidence levels, the percent changes in concentration from the intercept of the best-fit regression line were 3.8, 5.4, and 2.8 percent for each matrix, respectively.

Additional samples were analyzed (shown on Table 5-31 as 140 days) following the initial statistical evaluation for the 32-day experiment. Figure 5-18 shows the slope results and experimentally determined MHT's using all data for computations. The initial statistical evaluation test results for each matrix were:

Experiment	Matrix	CT (days)	LCL (days)
18	1	28.3	12.1
	2	32	32
	3	33.9	17.1

The slope test for Matrix 2 was not significantly different than a slope of zero. Statistical results using the additional data were:

Experiment	Matrix	CT (days)	LCL (days)
18	1	195.4	79.1
	2	76.7	37.5
	3	69.1	45.1

Therefore, the experimentally determined MHT's for the three matrices of the sulfide experiment are 32 days.

Table 5-30. Sulfide (mg/L) Results for Samples Analyzed at Time = 0.

MATRIX	EXPERIMENT	SAMPLE TYPE	MEAN (MG/L)	STD.DEV. (MG/L)
1	18	USUP	0.017	0.012
1	18	USP	0.038	0.034
2	18	USUP	0.010	0.000
2	18	USP	0.010	0.000
3	18	USUP	0.015	0.009
3	18	USP	0.035	0.001

USUP = UNSPIKED,UNPRESERVED
USP = UNSPIKED,PRESERVED

Table 5-31. Sulfide (mg/L) Raw Data and Summary Statistics for Experiment 18.

TIME	MATRIX 1			MATRIX 2			MATRIX 3		
	DATA	MEAN	S.D.	DATA	MEAN	S.D.	DATA	MEAN	S.D.
0	0.544	0.531	0.019	0.106	0.102	0.004	0.777	0.773	0.003
	0.541			0.097			0.771		
	0.509			0.102			0.771		
2	0.665	0.646	0.019	0.120	0.117	0.003	0.790	0.798	0.007
	0.646			0.113			0.798		
	0.626			0.118			0.805		
4	0.627	0.613	0.013	0.127	0.127	0.002	0.862	0.864	0.005
	0.609			0.125			0.870		
	0.602			0.129			0.862		
8	0.601	0.607	0.039	0.112	0.112	0.001	0.751	0.756	0.005
	0.571			0.112			0.756		
	0.648			0.114			0.762		
16	0.638	0.627	0.015	0.099	0.099	0.00003	0.862	0.856	0.008
	0.633			0.099			0.847		
	0.610			0.099			0.860		
32	0.640	0.649	0.014	0.120	0.122	0.004	0.867	0.864	0.008
	0.640			0.120			0.855		
	0.666			0.126			0.871		
140	0.542	0.541	0.011	0.116	0.131	0.013	0.890	0.897	0.012
	0.551			0.138			0.910		
	0.530			0.140			0.890		
	MEAN	S.D.	C.V.	MEAN	S.D	C.V.	MEAN	S.D.	C.V.
TOTAL	0.602	0.048	8.021	0.116	0.013	10.82	0.830	0.051	6.179

S.D. = Standard Deviation
C.V. = Coefficient of Variation (percent)
CONC = Concentration in mg/L

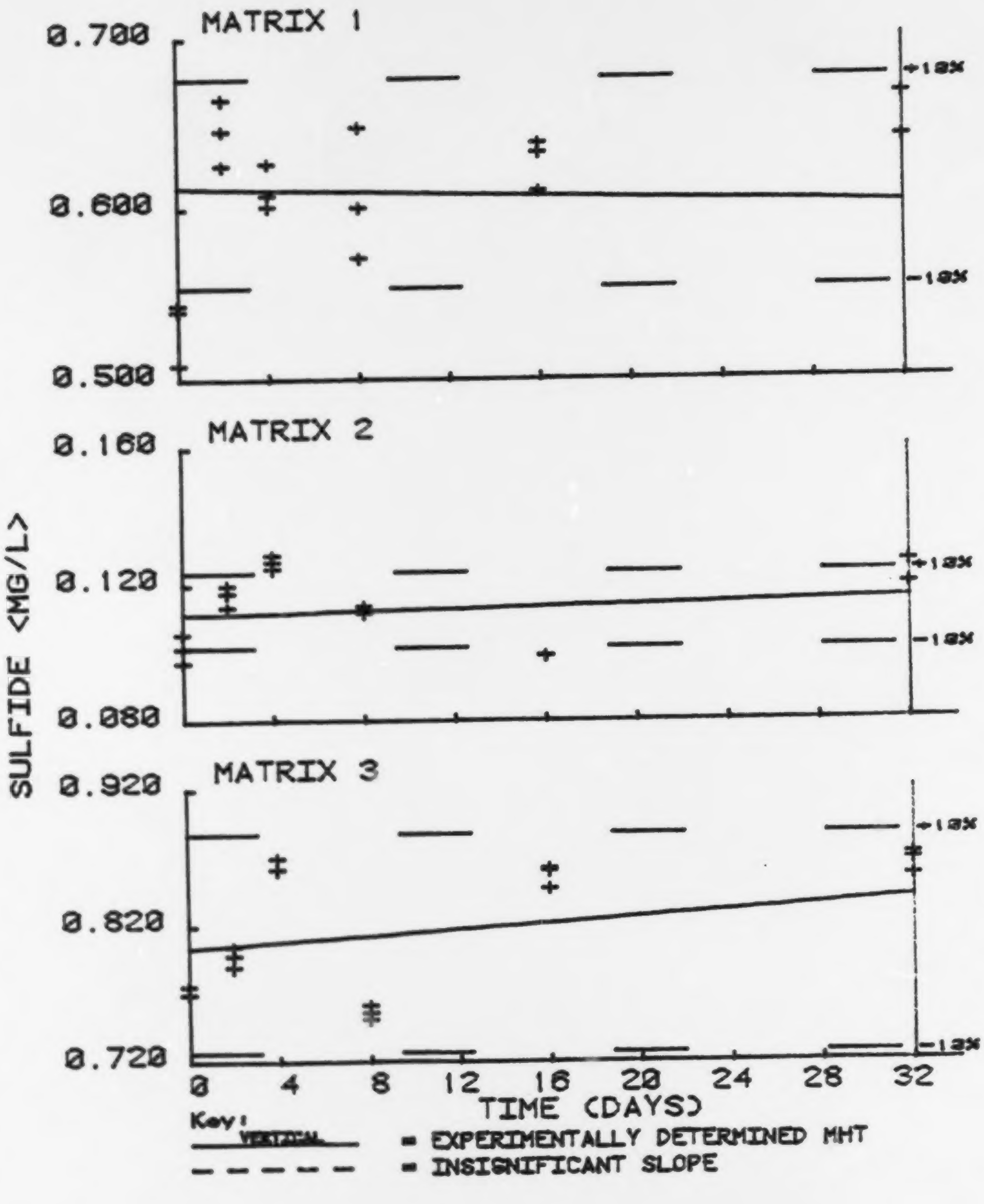


Figure 5-18. STATISTICAL RESULTS OF EXPERIMENT 18 FOR SULFIDE

REFERENCES

Barr, A.J. et al. 1979. SAS Users Guide: 1979 Edition. SAS Institute, Inc., Raleigh, North Carolina.

Carter, M.J. and Huston, M.T. 1978. Preservation of Phenolic Compounds in Wastewaters. Environmental Science and Technology, 12(3):309-312.

Finney, D.J. 1964. Statistical Method in Biological Assay. Charles Griffen and Co., London.

Gillespie, W.T. 1982. Laboratory Data Management System with Quality Control. American Laboratory, 14(2):106-111.

Hartley, H.O. 1950. The Maximum F-Ration as a Shortcut for Heterogeneity of Variance. Biometrika, 37:308-312.

Matsunaga, K., Konishi, S., and Nishimura, M. 1979. Errors Caused Prior to Measurement of Mercury in Natural Waters with Special Reference to Sea Water. Environmental Science and Technology, 13(1):63-65.

Mendenhall, W. and McClave, J.T. 1981. A Second Course in Business Statistics: Regression Analysis. Dellen Publishing Co., San Francisco.

Moore, S.R. 1980. Water Discharge Monitoring Requirements Under the NPDES Permits Program. EPA-600/4-81-033. U.S. Environmental Protection Agency, Cincinnati, Ohio.

Perkin Elmer. 1981. Analytical Methods for Atomic Absorption Spectroscopy Using the MHS Mercury/Hydride System. Instrument Division, Perkin Elmer Corporation, Norwalk, Connecticut.

Stephens, M.A. 1974. Use of the Kolmogorov-Smirnov, Cramer-Von Mises and Related Statistics Without Extensive Tables. Journal of the American Statistical Association, 69:730-737.

U.S. Environmental Protection Agency. 1978. Manual for the Interim Certification of Laboratories Involved in Analyzing Public Drinking Water Supplies; Criteria and Procedures. EPA-600/8-78-008.

U.S. Environmental Protection Agency. 1979a. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020.

U.S. Environmental Protection Agency. 1979b. Part III, Environmental Protection Agency: Guidelines Establishing Test Procedures for the Analysis of Pollutants; Proposed Regulations, Federal Register, 44((233):69464-69575. EPA-600/D-80-021.

U.S. Environmental Protection Agency. 1979c. Part IV, Environmental Protection Agency: Guidelines Establishing Test Procedures for the Analysis of Pollutants; Proposed Regulations; Correction, Federal Register, 44(244):75028-75052. EPA-600/D-80-022.

U.S. Environmental Protection Agency. 1980. Interim Guidelines and Specifications for Preparing Quality Assurance Project Plans. QAMS-005/80.

U.S. Environmental Protection Agency. 1983. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020.

Winter, J., Britton, P., Clements, H., and Kroner, R. 1977. EPA Method Study 8, Total Mercury in Water. EPA-600/4-77-012. U.S. Environmental Protection Agency, Cincinnati, Ohio.

APPENDIX

STATISTICAL PROGRAM DOCUMENTATION

The purpose of this appendix is to document the original EPA protocol and the computer programs used to implement the statistical protocol (see Section 4.7) for calculations of critical time (CT) and lower confidence limit (LCL). The original EPA protocol is presented in Table A-1. Two types of computer programs were used for the statistical protocol. The main program used the Statistical Analysis System (SAS) (Barr, 1979) programs to perform the entire protocol developed for the statistical evaluation. The other program, written in Fortran, was used to:

1. Verify the first-order model calculations for CT and LCL,
2. Prepare raw data and summary statistics tables for the final report, and
3. Prepare data files for graphics of statistical results for the final report.

Tables A-2 and A-3 present the SAS computer program and the Fortran program, respectively.

To calculate an MHT estimate, the CT is defined as that time at which the concentration is 90 percent of the initial concentration, and the initial concentration is best estimated by the intercept of the reaction rate model. For the zero-order model, the value of CT is obtained by solving:

$$0.9a_i = a_i + k_i\,(CT)$$

for

$$CT = \frac{-0.1a_i}{k_i} \qquad (1)$$

where: a_i = the intercept of the i^{th} matrix model, and
k_i = the reaction rate (slope) of the model.

For the first-order model, CT is found by solving:

$$\log\,(9a_i) = \log\,(a_i) + k_i(CT)$$

$$CT = \frac{\log(0.9a_i) - \log(a_i)}{k_i} = \frac{\log(0.9) + \log(a_i) - \log(a_i)}{k_i}$$

$$= \frac{\log(0.9)}{k_i} \qquad (2)$$

Thus, the CT value is independent of the initial concentration a_i, and depends only on the reaction rate k_i.

The lower 90-percent confidence limit must be calculated for each CT. To calculate this, for the zero-order model, recall Equation 1. Use Fieller's Theorem (Finney, 1964) to find an approximate variance for the ratio of two random variables ($0.1a_i$ and k_i in this case):

$$\mathrm{Var(CT)} = \frac{1}{k_i^2} \quad [\mathrm{Var}(-0.1a_i) + 2(\mathrm{CT})\ \mathrm{Cov}\ (-0.1a_i,k_i)$$

$$+ (\mathrm{CT})^2 \quad \mathrm{Var}(k_i)]$$

$$= \frac{1}{k_i^2}\ [0.01\ \mathrm{Var}(a_i) + 0.2(\mathrm{CT})\ \mathrm{Cov}(a_i,k_i) + (\mathrm{CT})^2\ \mathrm{Var}(k_i)]$$

The variances and covariances in the above formula can be derived from the variances and covariances of the regression coefficients.

When the model is first order, the formula for CT is Equation 2. Then, again using Fieller's Theorem (the numerator is constant in this case):

$$\mathrm{Var(CT)} = (\mathrm{CT})^2\ \mathrm{Var}(k_i)/k_i^2$$

The approximate lower 90 percent confidence limit is given by:

$$\mathrm{LCL} = \mathrm{CT} - 1.34\ \sqrt{\mathrm{Var(CT)}}$$

where the distribution of CT is assumed to be approximately normally distributed. This assumption is primarily dependent on the sample size, and the 18 measurements should be adequate to make the approximation reasonable.

Table A-1 Definition and Procedure for the Determination of the Maximum Holding Time 1.3

Definition

Maximum Holding Time (MHT) is that time at which a 10% change in the analyte concentration (C_{t10}) occurs and the precision of the method of measurement allows the 10% change to be statistically different than 0% change (C_{t0}) at the 90% confidence level. When the precision of the method is not sufficient to statistically discriminate a 10% change in the concentration from 0% change, then the maximum holding time is that time where the % change in the analyte concentration (C_{tn}) is statistically different than the concentration at zero time (C_{t0}) at the 90% confidence level.

Procedure

Determination of Maximum Holding Time

Sample Collection and Treatment

(1) All samples are replicated in triplicate (3).

(2) Each matrix shall be assessed at only one spiked anlyte concentration.

(3) The schedule of analyses shall be at time intervals of zero time, tx, 2tx, 4tx, 8tx, and 16tx where tx is recommended to be 2 days but shorter time frames can be accommodated where necessary. Zero time is the time of initial analysis of the spiked sample which shall not exceed 48 hours after the sample collection. Storage of the sample upon collection shall be 4°C.

(4) Each analyte shall be stored separately in a matrix or it must be proven or assumed that physical, chemical, or biochemical interactions are not important to the methodology.

Statistical Tests and Logic

(1) For each set of triplicate data calculate a sample mean and standard deviation.

(continued)

Table A-1 (continued)

(2) Convert each piece of data to a Z score using the sample mean and standard deviation.

(3) Arrange the Z score in a frequency table and conduct Chi-square Goodness of Fit test for normality.

(4) If the calculated Chi-square is larger than Chi-square in the tables, under 99% confidence, the data is nonnormal; go to step 15. Otherwise continue to step 5.

(5) Conduct Bartlett's test for homogeneity of sample variances. If the calculated F score is larger the $F_{0.99}$ in the table for the appropriate degrees of freedom, the data is inhomogeneous; go to step 15. Otherwise continue to step 6.

(6) Using regression analysis of the replicate concentrations with time, calculate k the reaction constant for zero order and first order reaction rate laws. Choose the order which gives the smallest linearity F ratio.

(7) Calculate the 90% confidence limits for k, the intercept a, and the limits corresponding to 0.9 C_{t0}. If the 90% confidence interval for k includes 0.0, then MHT = 16tx, where tx = time interval. STOP. Otherwise continue with the next step.

(8) If $F_0 > F_1$, go to step 10. Otherwise continue with the next step. F_0 and F_1 are the linearity F ratios for zero and first order kinetics.

(9) Calculate the lower 90% confidence limit (MHT_{LCL}) for the MHT. Go to step 11 using first order kinetics.

(10) Zero order knetics are appropriate to describe analyte behavior. Calculate the 90% lower confidence limit for maximum holding time (MHT_{LCL}).

Go to the next step.

(11) If LCL < 0, go to step 12. Otherwise:

$$MHT = \frac{\ln (C_{t10}) - \ln a}{k} \qquad F_1 < F_0$$

first order rate

or

(continued)

Table A-1 (continued)

$$MHT = \frac{C_{t10} - a}{k}$$ $F_0 < F_1$
zero order rate

(12) If $F_1 < F_0$

$$MHT \cong \frac{\ln C_{t10} - \ln a}{k} + \left| MHT_{LCL} \right|$$

or if $F_0 < F_1$

$$MHT \cong \frac{C_{t10} - a}{k} + \left| MHT_{LCL} \right|$$

STOP.

(13) The data was not normal or homogeneous k may still be calculated, but a nonparametric test such as the Kendall-slope method may be used to place confidence limits on k or, a transformation of the data may give normal and/or homogeneous data.

(14) Go to step 8 through 13 with the modified nonparametric confidence limits, or, go back to step one with the transformed data.

Assumptions

(1) It is assumed that the respective concentration spikes can be made with sufficiently small error.

(2) Samples are moderately stable.

(3) It is assumed that the maximum holding time is matrix dependent.

(4) All samples are subjected to the same temperature changes, and the temperature should be held as constant as possible.

(5) Normality of the pure error is an assumption of the statistical tests.

(6) Homogenity of the data is an assumption of the statistical tests.

(7) It is a preliminary but not a necessary assumption that the decay rate will be zero or first order.

TABLE A-2. SAS COMPUTER PROGRAM

```
==============================================================
//MAXHLD07 JOB (8000,1013,30,10,0),MCCLAVE,CLASS=2
/*PASSWORD
/*ROUTE  PRINT R18
// EXEC SAS
//SYSIN DD *
**************************************************************
           DATA INPUT ROUTINE
      EACH RECORD MUST CONTAIN MATRIX NUMBER, CONCENTRATION,
      EXPERIMENTAL TIME, AND REAL TIME (SEPARATED BY BLANKS).
      EXPERIMENTAL TIME IS USED FOR PRESENTING DATA IN A
      SIMPLER FORMAT FOR ALL THREE MATRICES WHEN REAL TIME
      IS DIFFERENT THAN EXPERIMENTAL TIME, REAL TIME IS
      USED FOR REGRESSION ANALYSIS TO CALCULATE MHT AND LCL.
**************************************************************
;
DATA IN;
INPUT MATRIX CONC TIME REALTIME;
LENGTH MATR $2.; MATR=MATRIX;
LOG_CONC=LOG(CONC);
CLASTIME=TIME;
LABEL CONC = ZERO ORDER
      TIME = TIME IN DAYS
       LOG_CONC = FIRST ORDER
      CLASTIME=QUALITATIVE TIME
;
CARDS;
/*INCLUDE 'FILENAME OF RAW DATA FILE'
;
**************************************************************
            THIS SECTION COMPUTES THE MEAN AND STANDARD
            DEVIATION FOR EACH MATRIX-TIME CELL.
            THE RAW DATA AND DESCRIPTIVE STATISTICS ARE
            THEN LISTED FOR EACH MATRIX.
**************************************************************
;
PROC SORT DATA=IN; BY MATRIX TIME;
OPTIONS LS=90 NONUMBER NODATE;
PROC MEANS DATA=IN NOPRINT MEAN STD; BY MATRIX TIME; VAR CONC;
     OUTPUT OUT=MHS MEAN=MN_CONC STD=STD_CONC;
PROC MEANS DATA=IN NOPRINT MEAN STD CV; BY MATRIX; VAR CONC;
     OUTPUT OUT=TOT MEAN=MN_CONC STD=STD_CONC CV=CV;
DATA PRT; MERGE IN MNS; BY MATRIX TIME;
DATA PRT; SET PRT TOT; IF TIME=, THEN TIME=999;
PROC SORT DATA=PRT; BY TIME MATRIX;
DATA PRT; SET PRT; BY TIME MATRIX;
     IF FIRST.MATRIX THEN NN=0; NN=NN+1; IF TIME=999 THEN NN=1;
     RETAIN NN;
PROC SORT DATA=PRT; BY TIME NN MATRIX;
DATA _NULL_; SET PRT; BY TIME NN MATRIX; FILE PRINT HEADER=HDR;
   C=(MATRIX-1)*24+8;
```

(CONTINUED)

TABLE A-2. (CONTINUED)

```
===============================================================================
   IF TIME LT 999 THEN DO;
      IF FIRST.TIME THEN PUT @1 TIME 4. @C CONC 7.3 MN_CONC 8.3 STD_CONC 8.3 @;
      ELSE IF NN=1 THEN PUT @C CONC 7.3 MN_CONC 8.3 STD_CONC 8.3 @;
      ELSE PUT @C CONC 7.3 @;
      IF LAST,NN THEN PUT; IF LAST,TIME THEN PUT //;
   END;
   ELSE DO;
           IF MATRIX=1 THEN PUT /// @11 'MEAN' @19 'S.D.' @27 'C.V.'
              @35 'MEAN' @43 'S.D.' @51 'C.V.' @59 'MEAN' @67 'S.D.' @75 'C.V.'
             @1 'TOTAL' MN_CONC 9.3 STD_CONC 8.3 CV 8.3 @;
           ELSE IF MATRIX=2 THEN PUT @32 MN_CONC 7.3 STD_CONC 8.3 CV 8.3 @;
           ELSE PUT @56 MN_CONC 7.3 STD_CONC 8.3 CV 8.3;
END; RETURN;
TITLE1 MAXIMUM HOLDING TIME PROJECT;
TITLE2 EXPERIMENT  'EXPERIMENT DESCRIPTION'
HDR: PUT //// @30 'RAW DATA AND SUMMARY STATISTICS'
     /// @1 'TIME' @17 'MATRIX 1' @41 'MATRIX 2' @65 'MATRIX 3' /
         @11 'DATA' @19 'MEAN' @27 'S.D.' @35 'DATA' @43 'MEAN' @51 'S.D.'
         @59 'DATA' @67 'MEAN' @75 'S.D.' //;
RETURN;
DATA DES; INPUT REPORT_1 $CHAR80.;
CARDS;

     THE FIRST STAGE OF THE MAXIMUM HOLDING TIME (MHT) ANALYSIS IS TO
CONDUCT AN ANALYSIS OF VARIANCE WHICH YIELDS THE RESIDUALS FOR EACH
MATRIX-TIME COMBINATION.  ESSENTIALLY, THE ANOVA JUST SUBTRACTS THE
CELL MEANS FROM EACH OBSERVATION.
     FOLLOWING THE ANOVA PRINTOUTS, A RESIDUAL ANALYSIS IS PERFORMED
FOR BOTH THE ZERO-ORDER AND FIRST-ORDER MODELS, WITH THE ZERO-ORDER
ANALYSIS ALWAYS APPEARING FIRST.  THE ANALYSIS IS PERFORMED ON STAN-
DARDIZED RESIDUALS (RESIDUAL DIVIDED BY STANDARD DEVIATION), AND CONSISTS
OF THE FOLLOWING COMPONENTS:

     1. A HISTOGRAM DEPICTING THE DISTRIBUTION OF THE RESIDUALS.  NOTE
        THAT THE HORIZONTAL AXIS IS THE NUMBER OF STANDARD DEVIATIONS,
        SO THAT THE VALUES OF -3 AND +3 SHOULD BE VERY RARE IF THE
        RESIDUALS ARE NORMALLY DISTRIBUTED.
     2. A DETAILED DISTRIBUTIONAL ANALYSIS OF THE STANDARDIZED RESIDUALS,
        INCLUDING SKEWNESS, KURTOSIS, A TEST OF NORMALITY, PERCENTILES,
        EXTREME VALUES, AND PROBABILITY PLOTS.  THESE PERMIT A MORE OBJEC-
        TIVE ANALYSIS OF THE RESIDUAL DISTRIBUTION THAN THE HISTOGRAMS.
        PARTICULAR ATTENTION SHOULD BE PAID TO THE SKEWNESS, EXTREME VALUES,
        AND THE TEST OF NORMALITY.  HOWEVER, THE REPLICATION OF THREE
        MEASUREMENTS PER CELL MAKES THE ASSUMPTION OF NORMALITY SLIGHTLY
        LESS CRUCIAL (CENTRAL LIMIT THEORY).  THE PRIMARY OBJECTIVE SHOULD
         BE TO ACHIEVE SYMMETRY OF THE RESIDUAL DISTRIBUTION.
     3. BOX PLOTS OF THE RESIDUALS FOR EACH MATRIX.  THESE PLOTS SHOW THE
        MEAN BY A '+' AND THE MEDIAN BY A DASHED HORIZONTAL LINE IN THE
        RECTANGULAR BOX.  THE ENDS OF THE BOX ARE THE 25TH AND 75TH PERCEN-
```

(CONTINUED)

TABLE A-2. (CONTINUED)

```
=========================================================================
        TILES, SO THAT THE LENGTH OF THE BOX IS THE INTERQUARTILE RANGE.
        THE VARIATION OF RESIDUALS CAN BE VISUALLY COMPARED BY OBSERVING
        THE RELATIVE LENGTHS OF THE BOXES.  OUTLIERS (EXTREME RESIDUALS)
        ARE INDICATED BY '0' OR '*'.
     4. BOX PLOTS OF THE RESIDUALS FOR EACH TIME.

     MAXIMUM HOLDING TIME DATA HAVE A TENDENCY TO BE LOGNORMALLY DISTRI-
BUTED, WHICH IS CONSISTENT WITH THE FIRST-ORDER MODEL.  THUS, THE RESIDUAL
DISTRIBUTION OF THE ZERO-ORDER MODEL SHOULD BE CLOSELY EXAMINED FOR RIGHT-
WARD SKEWNESS AND OUTLIERS.  THEIR PRESENCE INDICATES THAT THE FIRST-ORDER
MODEL MAY BE PREFERABLE.  A MORE FORMAL TEST OF NORMALITY IS THE 'PROB>D'
IN THE RESIDUAL DISTRIBUTION PRINTOUT.  IF THIS SIGNIFICANCE LEVEL IS SMALL
THE EVIDENCE IS THAT THE DISTRIBUTION IS NOT NORMAL.  THUS, LOOK AT THE
RESIDUAL CONCENTRATION TO CHECK NORMALITY, AND THE LOG RESIDUAL CONCENTRATION
TO CHECK LOGNORMALITY.  IN ADDITION, THE BOX PLOTS SHOULD BE USED TO CHECK
FOR HOMOGENEITY OF VARIANCE AND LACK OF OUTLIERS.
OPTIONS NOCENTER;
PROC PRINT DATA=DES; ID REPORT_1; FORMAT REPORT_1 $CHAR80.;
OPTIONS CENTER;
*****************************************************************
          THE FOLLOWING SECTION PERFORMS AN ANALYSIS OF
          VARIANCE THAT YIELDS THE RESIDUALS FOR EACH
          MATRIX-TIME COMBINATION.  THE RESIDUALS ARE THEN
          STANDARDIZED (DIVIDED BY THE STANDARD DEVIATION)
          AND ANALYZED FOR NORMALITY, EQUAL VARIANCE, OUTLIERS, ETC.
*****************************************************************

;
PROC GLM DATA=IN; CLASSES MATRIX TIME;
MODEL CONC LOG_CONC = MATRIX TIME MATRIX=TIME/ SS4;
OUTPUT OUT=RESID RESIDUAL=RES_CONC RES_LCON;
TITLE3 ANOVA FOR CONCENTRATION AND LOG TRANSFORM OF CONCENTRATION;
TITLE4 MATRIX AND TIME ARE FACTORS;
TITLE6 THIS ANOVA IS PERFORMED PRIMARILY TO OBTAIN THE RESIDUALS;
TITLE7 IN ORDER TO ENABLE AN ANALYSIS OF THEIR DISTRIBUTIONAL PROPERTIES;
PROC MEANS NOPRINT DATA=RESID STD; VAR RES_CONC RES_LCON;
     OUTPUT OUT=STD STD=STD_RCON STD_LCON;
DATA RESID; SET RESID; NN=1; DATA STD; SET STD; NN=1;
DATA RESID; MERGE RESID STD; BY NN;
     RES_CONC=RES_CONC/STD_RCON; RES_LCON=RES_LCON/STD_LCON;
FORMAT RES_CONC RES_LCON 4.;
   LABEL RES_CONC = STD, ZERO ORDER RESIDUAL
         RES_LCON = STD, FIRST ORDER RESIDUAL

;
PROC CHART DATA=RESID; VBAR RES_CONC / TYPE=PCT LEVELS=5;
TITLE9 HISTOGRAM OF STANDARDIZED RESIDUALS;
PROC CHART DATA=RESID; VBAR RES_LCON / TYPE=PCT LEVELS=5;
PROC UNIVARIATE PLOT NORMAL DATA=RESID; VAR RES_CONC RES_LCON;
TITLE9 DISTRIBUTIONAL PROPERTIES OF STANDARDIZED RESIDUALS;
PROC SORT DATA=RESID; BY MATRIX;
PROC BPLOT DATA=RESID; VAR RES_CONC RES_LCON; CLASS MATRIX;
```

(CONTINUED)

TABLE A-2. (CONTINUED)

```
==============================================================================

TITLE9 BOX PLOTS OF STANDARDIZED RESIDUALS BY MATRIX;
PROC SORT DATA=RESID; BY TIME;
PROC BPLOT DATA=RESID; VAR RES_CONC RES_LCON; CLASS TIME;
TITLE9 BOX PLOTS OF STANDARDIZED RESIDUALS BY TIME;
DATA DES; INPUT REPORT_2 $CHAR80.;
CARDS;
     THE NEXT STAGE OF THE MHT ANALYSIS IS TO DETERMINE WHETHER EITHER THE
ZERO- OR FIRST-ORDER MODEL PROVIDES A GOOD FIT TO THE DATA.  THERE ARE SIX
ANALYSES OF VARIANCE PERFORMED, TWO FOR EACH MATRIX-ORDER COMBINATION.
     THE MOST IMPORTANT ASPECT OF EACH ANALYSIS IS THE TEST OF THE 'LACK_FIT'
TERM.  THIS TEST, APPEARING IN THE LAST LINE OF EACH ANALYSIS, IS A TEST TO
DETERMINE WHETHER THE DATA INDICATE THAT HIGHER ORDER (CURVILINEAR) TERMS
ARE IMPORTANT.  IN OTHER WORDS, THIS TEST INDICATES WHETHER THE LINEAR FIT
IS ADEQUATE.  A SMALL SIGNIFICANCE LEVEL (LABELLED 'PR > F' ON THE PRINTOUT)
INDICATES A LACK OF LINEAR FIT, WHILE A LARGE SIGNIFICANCE LEVEL INDICATES
THAT THE LINEAR FIT MAY BE ADEQUATE (SPECIFICALLY, A LARGE SIGNIFICANCE LEVEL
MEANS THAT THE DATA PROVIDE INSUFFICIENT EVIDENCE TO REJECT THE LINEAR FIT
IN FAVOR OF A HIGHER ORDER).

   1. CASE 1       'PR > F' < .10        LACK OF FIT IS SIGNIFICANT
     IF THE TEST INDICATES A LACK OF FIT OF BOTH THE ZERO- AND FIRST-ORDER
MODEL, IT IS UNLIKELY THAT THE ESTIMATES OF MHT USING THESE MODELS ARE
RELIABLE.  ONE POSSIBLE IMPLICATION IS THAT THE ANALYTICAL ERROR IS SIGNIFI-
CANTLY LESS THAN THE EXPERIMENTAL ERROR ASSOCIATED WITH THE REPLICATES AT
DIFFERENT TIMES.  THIS COULD HAVE THE EFFECT OF INDICATING A SIGNIFICANT
LACK-OF-FIT, AND MEANS AT DIFFERENT TIMES MAY DIFFER IN A RANDOM, NON-LINEAR
PATTERN.  IN THIS CASE, AN EVALUATION OF THE INDIVIDUAL MEANS AT EACH TIME
SHOULD BE MADE.  THIS CAN BE ACCOMPLISHED BY COMPARING THE MEANS FOR EACH TIME,
PAYING SPECIAL ATTENTION TO THE PERCENT DIFFERENCE BETWEEN MEANS.  NOTE THAT
THE MEANS OF THE LOG TRANSFORM (FIRST-ORDER) MEANS MUST BE EXPONENTIATED TO
OBTAIN THE GEOMETRIC MEANS IN ORDER TO DETERMINE THE PERCENT DIFFERENCE.

   2.  CASE 2      'PR > F' > .10         LACK OF FIT NON-SIGNIFICANT
     IF THE LACK-OF-FIT TEST INDICATES NO LACK OF LINEAR FIT, THE USER SHOULD
PROCEED TO REPORT_4 AND EXAMINE THE TEST OF SLOPE (TIME) FOLLOWING THAT REPORT.
OPTIONS NOCENTER ;
PROC PRINT DATA=DES; ID REPORT_2; FORMAT REPORT_2 $CHAR80.;
OPTIONS CENTER;
TITLE2 EXPERIMENT  'EXPERIMENT DESCRIPTION'
PROC SORT DATA=IN; BY MATRIX;
DATA EOF; SET IN; RENAME TIME=LINEAR CLASTIME=LACK_FIT;
PROC GLM DATA=EOF; BY MATRIX; CLASSES LACK_FIT;
     MODEL CONC LOG_CONC = LINEAR LACK_FIT / SS1;
TITLE3 ANOVA FOR EACH MATRIX;
TITLE4 LINEAR LACK OF FIT TEST;
DATA DES; INPUT REPORT_3 $CHAR80.;
CARDS;
     THE OBJECTIVE OF THIS SECTION IS TO COMPARE THE MEANS AT EACH TIME FOR
EACH MATRIX USING DUNCAN'S PROCEDURE.  FIRST, THE PRELIMINARY ANOVA ANALYSIS
FOR THE ZERO- AND FIRST-ORDER MODELS FOR EACH MATRIX IS PRESENTED.  THEN
THE DUNCAN'S COMPARISONS ARE GIVEN FOR EACH ORDER.
```

(CONTINUED)

TABLE A-2. (CONTINUED)

```
===========================================================================
     THESE COMPARISONS WILL BE MOST USEFUL IN CASE THE PREVIOUS LACK OF FIT
TESTS INDICATE THAT NEITHER THE ZERO-ORDER NOR THE FIRST-ORDER MODEL PROVIDES
AN ADEQUATE FIT, I.E., THE LACK-OF-FIT SIGNIFICANCE LEVEL IS SMALL.  DUNCAN'S
PROCEDURE WILL COMPARE ALL MEANS AND INDICATE WHICH ARE SIGNIFICANTLY
DIFFERENT.  IF THE ANALYTICAL ERROR IS MUCH SMALLER THAN THE EXPERIMENTAL
ERROR, THE PATTERN REVEALED BY THE DUNCAN'S COMPARISONS WILL BE RANDOM.  ALSO,
THE PERCENT DIFFERENCE BETWEEN THE MEANS WILL BE SMALL, EVEN THOUGH THEY MAY
BE STATISTICALLY SIGNIFICANT.
OPTIONS NOCENTER;
TITLE2 EXPERIMENT  'EXPERIMENT DESCRIPTION'
PROC GLM DATA=IN; BY MATRIX; CLASSES TIME;
     MODEL CONC LOG_CONC = TIME / SS1;
     MEANS TIME / DEPONLY DUNCAN;
TITLE4 TIME COMPARISONS MADE USING DUNCAN''S METHOD;
DATA DES; INPUT REPORT_4 $CHAR80.;
CARDS;
     THE FOLLOWING STAGE OF THE ANALYSIS FITS THE ZERO- AND FIRST-ORDER
MODELS.  A SEPARATE MODEL IS FIT TO EACH MATRIX, SINCE PRELIMINARY STUDIES
SHOWED THAT THE SLOPES AND VARIANCES WERE RARELY HOMOGENEOUS ACROSS MATRICES.
THE TEST OF SLOPE (TIME) CAN BE USED TO DETERMINE WHETHER THE DATA INDICATE
THAT THERE IS A SIGNIFICANT DECREASING (OR INCREASING) TREND IN THE CONCEN-
TRATION OVER TIME.
     IF THE SLOPE'S SIGNIFICANCE LEVEL (LABELLED 'PROD > F' IN THE PRINTOUT) IS
LARGE THE IMPLICATION IS THAT THERE MAY BE NO LINEAR TREND, IF THE PREVIOUS
DUNCAN'S ANALYSIS SHOW ONLY RANDOM DIFFERENCES BETWEEN MEANS AT DIFFERENT
TIMES, THE BEST ESTIMATE OF THE MHT IS THE MAXIMUM TIME IN THE EXPERIMENT.
     IF THE SLOPE'S SIGNIFICANCE LEVEL IS SMALL, THE IMPLICATON IS THAT A
SIGNIFICANT TREND EXISTS, AND THE MHT ESTIMATES IN THE FOLLOWING SECTION ARE
RELIABLE.  THE ORDER TO BE USED SHOULD BE DETERMINED BY COMBINING THE RESULTS
OF THE SLOPE'S RESIDUAL ANALYSES, LACK-OF-FIT TESTS, AND THE SLOPE TESTS.
OPTIONS NOCENTER;
PROC PRINT DATA=DES; ID REPORT_4; FORMAT REPORT_4 $CHAR80.;
OPTIONS CENTER;
TITLE2 EXPERIMENT  'EXPERIMENT DESCRIPTION'
PROC REG DATA=IN OUTEST=EST COVOUT; BY MATRIX;
MODEL CONC LOG_CONC = REALTIME / P;
   OUTPUT OUT=MOD PREDICTED=PRD_CONC PRD_LCON;
   LABEL REALTIME = SLOPE;
TITLE 3 REGRESSION ANALYSES OF CONCENTRATION AND LOG CONCENTRATION VS. REAL TIME
TITLE4 ZERO- AND FIRST-ORDER REACTION RATE MODELS, RESPECTIVELY;
PROC SORT DATA=EST; BY LOG_CONC MATRIX;
DATA EST; SET EST;
   IF CONC=-1 THEN DO;
      IF _TYPE_='OLS' THEN DO;
         STD_COND=_SIGMA_;
        A=INTERCEP; K=REALTIME;
       END;
       IF _MODEL_='INTERCEP' THEN DO;
          VAR_A=INTERCEP; COV_AK=REALTIME;
       END;
```

(CONTINUED)

TABLE A-2. (CONTINUED)

```
=========================================================================
      IF _MODEL_='REALTIME' THEN DO;
         VAR_K=REALTIME;
        OUTPUT;
      END;
    END;
    ELSE DO;
       IF _TYPE_='OLS' THEN DO;
           A=INTERCEP; K=REALTIME;
           STD_LCON=_SIGMA_;
      END;
      IF _MODEL_='INTERCEP' THEN DO;
           VAR_A=INTERCEP; COV_AK=REALTIME;
      END;
      IF _MODEL_='REALTIME' THEN DO;
           VAR_K=REALTIME;
        OUTPUT;
      END;
    END;
RETAIN A K VAR_A VAR_K COV_AK
      STD_CONC STD_LCON;
KEEP A K VAR_A VAR_K COV_AK MATRIX CONC LOG_CONC STD_CONC STD_LCON;
PROC MEANS NOPRINT MEAN CSS N MAX DATA=IN; BY MATRIX; VAR TIME;
   OUTPUT OUT=TEMP MEAN=XBAR CSS=SSX N=N MAX=MAX;
***************************************************************
                  CALCULATION OF HUNTER LCL
***************************************************************
PROC SORT DATA=EST; BY MATRIX;
DATA EST; MERGE EST TEMP; BY MATRIX;
      DF=N-2;
      PROB=1-PROBF((K*K/VAR_K),1,DF);
      DF1=2; P1=.9; P2=.95; IF CONC=-1 THEN VAR=STD_CONC*STD_CONC;
                              ELSE VAR=STD_LCON*STD_LCON;
     F=FINV(P1,DF1,DF); CHISQ=CINV(P2,DF);
      CL_TERM=1.34*SQRT(DF*VAR/CHISQ);
      RATIO=VAR/SSX;
 IF K LT 0 THEN DO;
    IF CONC=-1 THEN MHT_CONC=.9*A; ELSE MHT_CONC=LOG(.9)+A;
 END;
 ELSE DO;
      IF CONC=-1 THEN MHT_CONC=1.1*A; ELSE MHT_CONC=LOG(1.1)+A;
 END;
 IF K LT 0 THEN DO;
    L2=MHT_CONC+1.34*CL_TERM;
    CHECK=A-SQRT(2*F*(VAR/N+XBAR*XBAR*RATIO));
    IF L2 GT CHECK THEN DO; LCL=0; RETURN; END;
 END;
 ELSE DO;
      L2=MHT_CONC-1.34*CL_TERM;
      CHECK=A+SQRT(2*F*(VAR/M+XBAR*XBAR*RATIO));
      IF L2 LT CHECK THEN DO; LCL=0; RETURN; END;
 END;
    TEMP=L2-A;
    AP=K*K-2*F*RATIO;
    BP=4*F*RATIO*XBAR-2*K*TEMP;
    CP=TEMP*TEMP-2*F*VAR/N-2*F*XBAR*XBAR*RATIO;
    T1=BP*BP-4*AP*CP;
```

(CONTINUED)

TABLE A-2. (CONTINUED)

```
==========================================================================
   IF T1 LT 0 THEN PUT;
           ELSE DO; T2=SQRT(T1);
                X1=(-BP+T2)/(2*AP); X2=(-BP-T2)/(2*AP);
                LCL=MIN(X1,X2);
           END;
DATA EST; SET EST;
  IF PROB GT .1 THEN DO;
     MHT=MAX; LCL_MHT=.; VAR_MHT=.; LCL_HNTR=.;
     IF CONC=-1 THEN DEPEND='CONCENTRATION'; ELSE DEPEND='LOG_CONC';
  END;
  ELSE DO;
   IF CONC=-1 THEN DO;
    DEPEND='CONCENTRATION';
IF ABS(1.75*SQRT(VAR_A)/A) LE 0.10 THEN P=0.1;ELSE P=ABS(1.75*SQRT(VAR_A)/A);
       IF K LT 0 THEN MHT=-P*A/K; ELSE MHT=P*A/K;
      VAR_MHT=(P*P*VAR_A+2*P*MHT*COV_AK+MHT*MHT*VAR_K)/(K*K);
      LCL_MHT=MHT-1.34*SQRT(VAR_MHT);
      LCL_HNTR=LCL;
   END;
   ELSE DO;
      DEPEND='LOG_CONC';
IF ABS(1.75*SQRT(VAR_A)) LT 0.10 THEN P=0.1;ELSE P=ABS(1.75*SQRT(VAR_A));
       IF K LT 0 THEN MHT=LOG(1-P)/K) ELSE MHT=LOG(1+P)/K;
     VAR_MHT=(MHT*MHT*VAR_K)/(K*K);
      LCL_MHT=MHT-1.34*SQRT(VAR_MHT);
      LCL_HNTR=LCL;
   END;
  END;
KEEP MHT VAR_MHT LCL_MHT DEPEND MATRIX LCL_HNTR PROB;
DATA DES; INPUT REPORT_5 $CHAR80.;
CARDS;
    THE MHT ESTIMATES CORRESPONDING TO THE ZERO- AND FIRST-ORDER MODELS
ARE LISTED ON THE FOLLOWING PAGE.  ALL THREE MATRICES ARE INCLUDED.  THESE
ESTIMATES REFLECT THE RESULT OF THE PREVIOUS SLOPE TEST.
     1. IF THE SLOPE IS NOT SIGNIFICANTLY DIFFERENT FROM ZERO, THE MHT IS
        ESTIMATED TO BE THE MAXIMUM TIME IN THE EXPERIMENT.  NO LOWER
        CONFIDENCE LIMIT IS ESTIMATED.
     2. IF THE SLOPE IS SIGNIFICANT, THE MHT ESTIMATE AND LOWER CONFIDENCE
        LEVEL ARE CALCULATED, AND SHOULD BE VALID.
    TWO METHODS ARE USED TO OBTAIN A LOWER 90% CONFIDENCE LIMIT FOR THE MHT;
FIELLER'S (LCL_MHT) AND HUNTER'S (LCL_HNTR).  FIELLER'S METHOD IS PROBABLY
MORE APPROPRIATE IN THIS CASE, BECAUSE IT IS DERIVED BY APPROXIMATING THE
VARIANCE OF THE MHT ESTIMATOR, HUNTER'S METHOD WAS DEVELOPED FOR ERRORS
ASSOCIATED WITH THE REPEATED USAGE OF A CALIBRATION LINE.  IN THE PRESENT
APPLICATION THE LINE IS BEING USED ONLY TO ESTIMATE THE MHT.
OPTIONS NOCENTER;
PROC PRINT DATA=DES; ID REPORT_5; FORMAT REPORT_5 $CHAR80.;
OPTIONS CENTER;
TITLE2 EXPERIMENT 'EXPERIMENT DESCRIPTION'
PROC SORT DATA=EST; BY DEPEND MATRIX;
DATA EST; SET EST;
     IF . LT LCL_MHT LT 0 THEN LCL_MHT=0;
     IF . LT LCL_HNTR LT 0 THEN LCL_HNTR=0;
     IF PROB GT .1 THEN SLOPE='NON-SIGNIFICANT'; ELSE SLOPE='SIGNIFICANT';
PROC PRINT DATA=EST; VAR DEPEND MATRIX SLOPE MHT VAR_MHT LCL_MHT LCL_HNTR;
    FORMAT MHT VAR_MHT LCL_MHT LCL_HNTR 10.1;
TITLE3 MAXIMUM HOLDING TIME ESTIMATE AND LOWER 90% CONFIDENCE LIMIT ;
TITLE4 VALUE IS GIVEN FOR EACH MATRIX,AND FOR BOTH ZERO-AND FIRST-ORDER MODELS;
TITLE5 BOTH FIELLER''S AND HUNTER''S METHODS ARE USED TO OBTAIN LOWER CL;
TITLE6 IF THE SLOPE IS NOT SIGNIFICANTLY DIFFERENT FROM ZERO, MHT = MAX, TIME OF
       EXPERXIMENT;
==========================================================================
```

TABLE A-3. FORTRAN PROGRAM

```
C   S_HOLDTIME     TOM GILLESPIE 3/8/82
C
$INSERT SYSCOM>A$KEYS
      IMPLICIT INTEGER*4 (K)
      LOGICAL LOG,LOG2
      REAL*8 TX2,TX,TXY,TY,TY2,TSSX,RFIT,RMSE
C
      COMMON /HTPLOT/ INAME(20),NPTS,RCONC(51),RGTIME(51),RMHT,RLCL,
     *           AINT,SLOPE
C   RECORD LENGTH= 437 WORDS = 872 BYTES
C      INAME(20)= ANALYSIS TITLE        NPTS=# OF VALUES
C      RCONC(102)=CONCENTRATIONS        RGTIME(102)=GROUP TIMES
C      RMHT= MAXIMUM HOLDING TIME       RLCL= LOWER CONFIDENCE LIMIT
C      NATURAL LOG(CONC) = AINT  + SLOPE * TIME
C
      COMMON /EXIT/ IEXSW,NUL
      COMMON /PANDH/IPROG(5),IHAND(10)
C
      DIMENSION RMEAN(250),RSD(250),RG1(250),RDATA(250),TIME(250),
     *          RTIME(250),ICARD(40),RDUM(10),
     *          KSRT(250),KPT(250),N2RX(3),R2X2(3),R2X(3),
     *          STAD(3),AV(3),CV(3),INFILE(10),JPOS(3,5),ITME(10),
     *          JTNAME(3),IPRT(2),FCRIT(50),TCRIT(30)
C
      DATA IPROG /'HO','LD','TI','ME','  '/
      DATA FCRIT/39.86,8.53,5.54,4.54,4.06,3.78,3.59,3.46,3.36,3.29,
     *            3.23,3.18,3.14,3.10,3.07,3.05,3.03,3.01,2.99,2.97,
     *            2.96,2.95,2.94,2.93,2.92,2.91,2.90,2.89,2.89,2.88,
     *            2.88,2.87,2.87,2.86,2.86,2.86,2.85,2.85,2.84,2.84,
     *            2.84,2.83,2.83,2.82,2.82,2.81,2.81,2.80,2.80,2.79/
C
      DATA TCRIT/6.314,2.920,2.353,2.132,2.015,1.943,1.895,1.860,
     *     1.833,1.812,1.796,1.782,1.771,1.761,1.753,1.746,1.740,
     *     1.734,1.729,1.725,1.721,1.717,1.714,1.711,1.708,1.706,
     *     1.703,1.701,1.699,1.697/
C
      ICONPS=2
      IGRPS=1
      ITMPS=3
      IRTMPS=4
      INUMP=4  /* # OF PARAMETERS
      CALL TNOU('PLOT OUTPUT FILE IS D_PLOT',26)
      LOG2=  OPNV$A(A$WRIT+A$SAMF,'D_PLOT',6,4,A$OVAP,2,2)
      CALL ATTDEV(8,8,4,437)
```

(CONTINUED)

Table A-3. (Continued)

```
=====================================================================
9     WRITE(1,10) IGRPS,ICONPS,ITMPS,IRTMPS,INUMP
10    FORMAT(I3,' = MATRIX #',/,I3,' = CONCENTRATION',/,I3,
     *  ' = TIME',/,I3,' = REAL TIME',/,I3,' = # OF VARIABLES',/
     *  '@ TO RESPECIFY PARAMETERS')
      CALL I2VL(IDUM)
      NRX=0
      RX=0
      RX2=0
      CALL IZERO(N2RX,3)
      CALL ZERO(R2X2,3)
      CALL ZERO(R2X,3)
      IF(IEXSW.NE.0) GO TO 100
      CALL I2VLM(IGRPS,'MATRIX POSITION ',16)
      CALL I2VLM(ICONPS,'CONC POS',8)
      CALL I2VLM(ITMPS,'TIME POS',8)
      CALL I2VLM(IRTMPS,'REAL TIME POS ',14)
      CALL I2VLMT(INUMP,'# OF PARAMETERS?',16)
100   CALL MSTRG('SPSS FILE NAME, OR @ TO FINISH',30,INFILE,10)
      IF(IEXSW.EQ.0) GO TO 999
      LOG=OPEN$A(A$READ+A$SAMF,INFILE,20,1)
      IF(.NOT.LOG) CALL TNOU('NO FILE',7)
      IF(.NOT.LOG) GO TO 100
      CALL TEMP$A(A$SAMF,JTNAME,6,2)
      IPRT(1)=:3401
      CALL SATR$$(K$PROT,JTNAME,6,IPRT(1),IERR)
      CALL ATTDEV(6,8,2,66)
      IFLE=6
      CALL TNOUA('OUTPUT IN FILE:',15)
      CALL TNOU(JTNAME,6)
      ICASE=1 /* COUNTER FOR # OF CASES
      IDATA=0 /* SET >= 1 FOR READING INPUT DATA (FLAG)
      ICNT=0 /* COUNTER FOR VARIABLES WITHIN EACH CASE
      DO 200 I=1,1000
        DO 115 J=1,10
115       RDUM(J)=-99999.
        CALL RDLIN$(1,ICARD,40,IERR)
        IF(IERR.NE.0) GO TO 250
        IF(IDATA.LT.1) GO TO 150    /* IF NOT A INPUT DATA CARD
        IF(ICARD(1).EQ.'FI') GO TO 250
        DECODE(80,*,ICARD,ERR=118) RDUM
118     IF(RDUM(1).LT.-1000) GO TO 200
        DO 125 J=1,10
          IF(RDUM(J).LT.-1000.) GO TO 125
          ICNT=ICNT+1
          IF(ICNT.GT.INUMP) ICASE=ICASE+1
```

(CONTINUED)

Table A-3. (Continued)

```
=================================================================
          IF(ICNT.GT.INUMP) ICNT=1
          IF(ICNT.EQ.IGRPS) RG1(ICASE)=RDUM(J)
          IF(ICNT.EQ.ICONPS) RDATA(ICASE)=RDUM(J)
          IF(ICNT.EQ.ITMPS) TIME(ICASE)=RDUM(J)
          IF(ICNT.EQ.IRTMPS) RTIME(ICASE)=RDUM(J)
125     CONTINUE
130   CONTINUE
        GO TO 200
150     IF(ICARD(1).EQ.'RE'.AND.ICARD(2).EQ.'AD') IDATA=1
        IF(ICARD(1).NE.'  ') ILAB=0
        IF(ICARD(3).EQ.'LA'.AND.ICARD(4).EQ.'BE') ILAB=1
        IF(ILAB.LT.1) GO TO 200
        IF(ICONPS.EQ.ILAB) CALL MSUB$A(ICARD,80,16,55,INAME,40,1,40)
        ILAB=ILAB+1
200   CONTINUE
250   CONTINUE
      CALL MSTRGT('VARIABLE NAME ',14,INAME,20)
      IST=1
      ILAST=RG1(1)
      RX=0
      RX2=0
      NRX=0
      DO 300 I=1,ICASE
        KPT(I)=I
300     KSRT(I)=TIME(I)*1000 + RG1(I)
      CALL SORTIT(KSRT,KPT,ICASE)
      ITN=0
      ITC=-1
      DO 210 I=1,ICASE
        IT=TIME(KPT(I))
        IF(ITC.EQ.IT) GO TO 210
        ITC=IT
        ITN=ITN+1
        ITME(ITN)=IT
210   CONTINUE
211   FORMAT('TIMES =',10I7,/)
      WRITE(1,211) (ITME(I),I=1,ITN)
      IH=0
      IL=1
      IF=1
      WRITE(IFLE,252) INAME
      DO 400 J=1,ICASE
        I=KPT(J)
        IF(RDATA(I).LT.-1000.) WRITE(1,251)
```

(CONTINUED)

Table A-3. (Continued)

```
=====================================================================
251     FORMAT('ERR ON READING INPUT DATA')
        IF(J.NE.1) IL=J-1
        IF(KSRT(I).EQ.KSRT(KPT(J-1)))  GO TO 350
        AVG=0.0
        STD=0.0
        IF(NRX.GT.0) AVG=RX/NRX
        STD=NRX*AVG**2+RX2-2*AVG*RX
        IF(NRX.GT.1) STD=SQRT(STD/(NRX-1))
      IF(NRX.LT.2) STD=0.0
        IH=1
252   FORMAT(/,T15,'RAW DATA AND SUMMARY STATISTICS',5X,20A2,
     *  ///,'TIME',
     *   T17,'MATRIX 1',T41,'MATRIX 2',T65,'MATRIX 3',/,T11,'DATA',
     *   T19,'MEAN',T27,'S.D.',T35,'DATA',T43,'MEAN',T51,'S.D.',T59,
     *   'DATA',T67,'MEAN',T75,'S.D.',///)
253   FORMAT(6X,4A2,T31,4A2,T55,4A2)
254   FORMAT(I4,2X,36A2)
255   FORMAT(//,T11,'MEAN',T19,'S.D.',T27,'C.V.',T35,'MEAN',T43,
     *   'S.D',T51,'C.V.',T59,'MEAN',T67,'S.D.',T75,'C.V.')
256   FORMAT('TOTAL ',36A2)
257   FORMAT(//)
        DO 320 JK=IF,IL
          RMEAN(KPT(JK))=AVG
          RSD(KPT(JK))=STD
320   CONTINUE
        IF(J.GT.ICASE) GO TO 405
      NRX=0
      RX2=0.
      RX=0.
        IF=J
350   IF(RDATA(I).EQ.-999.) GO TO 400
      R2=RDATA(I)**2
      NRX=NRX+1
      RX2=RX2+R2
      RX=RX+RDATA(I)
      IF(RG1(I).GT.3.OR.RG1(I).LT.1) GO TO 390
      IS=RG1(I)
      N2RX(IS)=N2RX(IS)+1
      R2X2(IS)=R2X2(IS)+R2
      R2X(IS)=R2X(IS)+RDATA(I)
      GO TO 400
390   WRITE(1,391) RG1(I)
391   FORMAT('BAD GROUP # ',F15.3)
```

(CONTINUED)

Table A-3. (Continued)

```
======================================================================

400    CONTINUE
         AVG=0.0
         STD=0.0
         IF(NRX.GT.0) AVG=RX/NRX
         STD=NRX*AVG**2+RX2-2*AVG*RX
         IF(NRX.GT.1) STD=SQRT(STD/(NRX-1))
       IF(NRX.LT.2) STD=0.0
         DO 404 JK=IF,IL
           RMEAN(KPT(JK))=AVG
           RSD(KPT(JK))=STD
404    CONTINUE
405    RDATA(ICASE+1)=-999.
       RMEAN(ICASE+1)=-999.
       RSD(ICASE+1)=-999.
       KPT(ICASE+1)=ICASE+1
406    FORMAT('KSRT=',9I7,/)
407    FORMAT('KPT=',9I7,/)
       DO 500 J=1,ITN /* LOOP FOR EACH TIME
         CALL FILL$A(ICARD,80,' ')
         L1='OK'
         DO 450 JJ=1,3 /* LOOP FOR EACH GROUP
           KF=ITME(J)*1000. + JJ /* CALC SEARCH KEY
           DO 420 JA=1,ICASE  /* SEARCH CASES FOR CORRECT KEY
420        IF(KF.EQ.KSRT(JA)) GO TO 425
           JA=ICASE+1
425        JPOS(JJ,1)=KPT(JA)
           JPOS(JJ,2)=KPT(JA+1)
           IF(KSRT(JA+1).NE.KSRT(JA).OR.JA+1.GT.ICASE) JPOS(JJ,2)=ICASE+1
           JPOS(JJ,3)=KPT(JA+2)
           IF(KSRT(JA+2).NE.KSRT(JA).OR.JA+2.GT.ICASE) JPOS(JJ,3)=ICASE+1
           JPOS(JJ,4)=KPT(JA+3)
           IF(KSRT(JA+3).NE.KSRT(JA).OR.JA+3.GT.ICASE) JPOS(JJ,4)=ICASE+1
           JPOS(JJ,5)=KPT(JA+4)
           IF(KSRT(JA+4).NE.KSRT(JA).OR.JA+4.GT.ICASE) JPOS(JJ,5)=ICASE+1
450       CONTINUE
          DO 475 JL=1,5   /* LIST CASES WITHIN EACH CELL
            IF(JL.EQ.1) GO TO 460
          CALL SFIG2(RDATA(JPOS(1,JL)),L1,4,3,ICARD(1))
          CALL SFIG2(RDATA(JPOS(2,JL)),L1,4,3,ICARD(5))
          CALL SFIG2(RDATA(JPOS(3,JL)),L1,4,3,ICARD(9))
            WRITE(IFLE,253) (ICARD(LL),LL=1,12)
            GO TO 475
460       CALL SFIG2(RDATA(JPOS(1,1)),L1,4,3,ICARD(1))
          CALL SFIG2(RDATA(JPOS(2,1)),L1,4,3,ICARD(13))
          CALL SFIG2(RDATA(JPOS(3,1)),L1,4,3,ICARD(25))
```

(CONTINUED)

Table A-3. (Continued)

```
=================================================================
        CALL SFIG2(RMEAN(JPOS(1,1)),L1,4,3,ICARD(5))
        CALL SFIG2(RSD(JPOS(1,1)),L1,4,3,ICARD(9))
        CALL SFIG2(RMEAN(JPOS(2,1)),L1,4,3,ICARD(17))
        CALL SFIG2(RSD(JPOS(2,1)),L1,4,3,ICARD(21))
        CALL SFIG2(RMEAN(JPOS(3,1)),L1,4,3,ICARD(29))
        CALL SFIG2(RSD(JPOS(3,1)),L1,4,3,ICARD(33))
        WRITE(IFLE,254) ITME(J),(ICARD(LL),LL=1,36)
475        CONTINUE
500    CONTINUE
       DO 600 I=1,3
         IF(N2RX(I).GT.0) AV(I)=R2X(I)/N2RX(I)
         STAD(I)=N2RX(I)*AV(I)**2 + R2X2(I) - 2*AV(I)*R2X(I)
         IF(N2RX(I).GT.1) STAD(I)=SQRT(STAD(I)/(N2RX(I)-1))
         IF(N2RX(I).LT.2) STAD(I)=0.0
         IF(AV(I).NE.0.0) CV(I)=100*STAD(I)/AV(I)
600    CONTINUE
       WRITE(IFLE,255)
         CALL SFIG2(AV(1),L1,4,3,ICARD(1))
         CALL SFIG2(STAD(1),L1,4,3,ICARD(5))
         CALL SFIG2(CV(1),L1,4,3,ICARD(9))
         CALL SFIG2(AV(2),L1,4,3,ICARD(13))
         CALL SFIG2(STAD(2),L1,4,3,ICARD(17))
         CALL SFIG2(CV(2),L1,4,3,ICARD(21))
         CALL SFIG2(AV(3),L1,4,3,ICARD(25))
         CALL SFIG2(STAD(3),L1,4,3,ICARD(29))
         CALL SFIG2(CV(3),L1,4,3,ICARD(33))
       WRITE(IFLE,256) (ICARD(LL),LL=1,36)
C
C   CALCULATE SCALE FOR PLOTS
       CALL SCALE(TIME,8.0,ICASE,1)
C
C
C      COMPUTE LINEAR REGRESSION EQUATION
C
611    FORMAT(//,T10,'REGRESSION OF LN_CONCENTRATION WITH TIME FOR ',
     *     20A2)
       CALL TOF
       WRITE(IFLE,611) INAME
       TX=0.0
       TY=0.0
       TX2=0.0
       TXY=0.
       INM=0
       IF=1
       KCHK=RG1(1)
```

(CONTINUED)

Table A-3. (Continued)

```
======================================================================
      DO 800 J=1,ICASE
        K2CHK=RG1(J)
        I=J
        IF(K2CHK.EQ.KCHK) GO TO 650
616     IF(INM.LT.2) GO TO 625
        XBAR=TX/INM
        YBAR=TY/INM
        SX2=TX2 - 2*XBAR*TX + INM*XBAR**2
        SXY=INM*XBAR*YBAR+TXY-XBAR*TY-YBAR*TX
        SLOPE=SXY/SX2
        AINT=YBAR-SLOPE*XBAR
621     FORMAT(//,' MATRIX #',I3,//,' SLOPE=',F12.6,'  INTERCEPT=',
     *             F12.6)
        WRITE(IFLE,621) KCHK,SLOPE,AINT
        RMHT=ITME(ITN)
        RMSE=0.0
        IL=I-1
        IF(I.EQ.ICASE) IL=IL+1
        TSSX=0.0
        IDF=IL-IF+1
        TYBAR=TY/IDF
        TXBAR=TX/IDF
        SSREG=0
      SSX1=TX2-TX**2/IDF
      SXY1=TXY-TX*TY/IDF
      SY1=(TY2-TY**2/IDF) - SXY1**2/SSX1
      SY1=SY1/(IDF-2)
      IF(SY1.LT.0) WRITE(1,617) J,SY1
617   FORMAT('SY1 FOR CASE #',I3,' =',F15.7)
      IF(SY1.GE.0) SY1=SQRT(SY1)
      IF(SY1.LT.0) SY1=0
      RP=SQRT(1.0/IDF + TXBAR**2/SSX1)
      RP=TCRIT(IDF-2)*SY1*RP
      SGP=0.1
      IF (RP.GE.1) RP=0.999
      IF (RP.GT.0.1) SGP=RP
        IF(SLOPE.LT.0) RMHT=ALOG(1.0-SGP)/SLOPE
        IF(SLOPE.GT.0) RMHT=ALOG(1.0+SGP)/SLOPE
        DO 6205 L=IF,IL
          RFIT= AINT + SLOPE * RTIME(L) /* CALCULATE PRED LN_CONC
          SSREG=SSREG+(RFIT-TYBAR)**2
          RFIT2=2.71828**RFIT   /* CONVERT FROM LOG FOR DISPLAY OF CONC.
          WRITE(IFLE,6203) RDATA(L),RFIT2
          RFIT=(ALOG(RDATA(L))-RFIT)**2 /* SUBTRACT PRED CONC FROM REAL
          RMSE=RMSE+RFIT  /* SUM SQUARE OF ERROR
```

(CONTINUED)

Table A-3. (Continued)

```
================================================================================
          TSSX=TSSX+(RTIME(L)-TXBAR)**2
6203      FORMAT('ACTUAL CONC=',F10.4,' PREDICTED CONC=',F10.4)
6205    CONTINUE
        RMSE=RMSE/(IDF-2)   /* CALCULATE MSE BY DIVIDING DEGREES FREEDOM
          TXBAR=TX/IDF   /* AVERAGE TIME
        VARK=RMSE/TSSX
        VARMHT=(RMHT**2)*VARK/SLOPE**2
        RLCL= RMHT - 1.34*SQRT(VARMHT)
62051     FORMAT('MSE=',F12.6,' VAR_K=',F12.9,' VAR_MHT=',F12.4)
        WRITE(IFLE,62051) RMSE,VARK,VARMHT
        F=SSREG/RMSE
        ID2=IDF-2
        IF(ID2.GT.50) ID2=50
         WRITE(IFLE,622) RMHT , RLCL,F,ID2,FCRIT(ID2)
622      FORMAT('MAXIMUM HOLD TIME=',F15.1,' LOWER CONFIDENCE LIMIT=',
     *    F15.2,/,' F-TEST=',F15.3,' DEGREES OF FREEDOM = 1,',I3,
     *   '  F-CRITERIA (0.90)=',F5.2)
        WRITE(IFLE,623) RP
623     FORMAT('SIGNIFIGANT CONCENTRATION DIFFERENCE IS AT',F10.4)
        ISSL=0
        IF(FCRIT(ID2).GT.F) ISSL=1
          KCHK=K2CHK
625       IC=0
          ALINT=2.71828**AINT
          RDUM(1)=1.1*ALINT
          RDUM(2)=0.9*ALINT
          DO 630 JJ=IF,IL
             IC=IC+1
             RCONC(IC)=RDATA(JJ)
             RGTIME(IC)=TIME(JJ)
              IF(RDUM(1).LT.RCONC(IC)) RDUM(1)=RCONC(IC)
              IF(RDUM(2).GT.RCONC(IC)) RDUM(2)=RCONC(IC)
630       CONTINUE
          NPTS=IL-IF+1
          CALL SCALE(RDUM,2.0,2,1)
          RCONC(NPTS+1)=RDUM(3)
          RCONC(NPTS+2)=RDUM(4)
645     RGTIME(NPTS+1)=TIME(ICASE+1)
        RGTIME(NPTS+2)=TIME(ICASE+2)
        RTMAX=RGTIME(1)
        DO 3102 IH=1,ICASE
             IF(TIME(IH).GT.RTMAX) RTMAX=RGTIME(IH)
3102    CONTINUE
        IF(0.0.GT.RMHT) RMHT=0.0
        IF(RTMAX.LT.RMHT) RMHT=RTMAX
        IF(0.0.GT.RLCL) RLCL=0.0
        IF(RTMAX.LT.RMHT) RLCL=RTMAX
```

(CONTINUED)

Table A-3. (Continued)

```
============================================================
     R2LCL=RLCL
     IF(ISSL.EQ.1) R2LCL=-999.
       IF(LOG2) WRITE(8,6301) INAME,NPTS,RCONC,RGTIME,RMHT,R2LCL,AINT,
    *               SLOPE
       TX=0.
6301  FORMAT(20A2,/,I5,/,17(6F12.4,/),4F17.8)
       TY=0.
       TY2=0.0
       TX2=0.
       TXY=0.0
       INM=0
       IF=I
       IF(J.EQ.ICASE) GO TO 805
650    IF(RDATA(I).EQ.-999.) GO TO 800
       TX=TX+RTIME(I)
       TX2=TX2 + RTIME(I)**2
       TY=TY + ALOG(RDATA(I))    /*  NATURAL LN
       TXY=TXY+RTIME(I)*ALOG(RDATA(I))
       TY2=TY2+ALOG(RDATA(I))**2
       INM=INM+1
       IF(J.EQ.ICASE) GO TO 616
800   CONTINUE
805   CONTINUE
9999  CALL CLOS$A(1)
      CALL CLOS$A(2)
      GO TO 9
999   CALL CLOS$A(1)
      CALL CLOS$A(2)
      CALL CLOS$A(4)
      STOP
      END
      SUBROUTINE SFIG2(RCONC,IPSTAT,IPSFG,IPDPT,ISTR)
      DOUBLE PRECISION RRDOUB
      IMPLICIT INTEGER*4 (K)
      DIMENSION ISTR(4),RV(1)
 $INSERT SYSCOM>A$KEYS
      IDPT=IPDPT
      ISTAT=IPSTAT
      IF(RCONC.LE.0.0) ISTAT='BK'
      IF (ISTAT.EQ.'@@') GO TO 400
      IF(ISTAT.NE.'OK'.AND.ISTAT.NE.'>'.AND.ISTAT.NE.'<'.
     1  AND.ISTAT.NE.'*'.AND.ISTAT.NE.' '.AND.ISTAT.NE.'*>'.AND.ISTAT
     2  .NE.'*<') GO TO 300
```

(CONTINUED)

Table A-3. (Continued)

```
==================================================================
      ISFIG=1
      IZERO=0
      R10=10
      RRDOUB=RCONC
      IF(IPSFG.LT.2) IPSFG=2
      CONC=ABS(RCONC)
      IF (CONC.EQ.0) GO TO 5
2      KEXP =-IDPT
      RTTT=R10**KEXP
       IF(RTTT.LE.CONC) GO TO 5
       IDPT = IDPT + 1
       GO TO 2
5     DO 10 I=1,10
        KEXP=I-IDPT
        IF(R10**KEXP.GT.CONC) GO TO 20
         ISFIG=ISFIG+1
10    CONTINUE
20    IF(ISFIG.LE.IPSFG) GO TO 110
      IF(IDPT.LE.0) GO TO 30
      ISFIG=ISFIG-1
      IDPT=IDPT-1
      GO TO 20
30    IZERO=IZERO+1
      ISFIG=ISFIG-1
      IF(ISFIG.GT.IPSFG) GO TO 30
      CONC=0.5+CONC/R10**IZERO
      CONC=AINT(CONC)
      RRDOUB=DBLE(CONC)
      RRDOUB=RRDOUB*R10**IZERO
110   CALL ENCD$A(ISTR,8,IDPT,RRDOUB)
      IF(ISTAT.EQ.'<'.OR.ISTAT.EQ.'>'.OR.ISTAT.EQ.'*'.OR.ISTAT.EQ.'*>'.
     1  OR.ISTAT.EQ.'*<') GO TO 200
      RETURN
200   CALL JSTR$A(A$LEFT,ISTR,8)
      ILEN=NLEN$A(ISTR,8)
      CALL JSTR$A(A$RGHT,ISTR,8)
      IF(ILEN.GE.8)CALL MSUB$A('********',8,1,8,ISTR,8,1,8)
      IF(ILEN.GE.10) ILEN=9
      ILN2=ILEN
      ILN=1
      IF(ISTAT.NE.'*>'.AND.ISTAT.NE.'*<') GO TO 222
          ILEN=ILEN+1
      ILN2=ILEN-1
      ILN=2
222   CALL MSUB$A(ISTAT,ILN,1,ILN,ISTR,8,8-ILEN,8-ILN2)
      RETURN
```

(CONTINUED)

Table A-3. (Continued)

```
======================================================================

300    CALL FILL$A(ISTR,8,' ')
       IF(ISTAT.EQ.'BK')GO TO 310
       ISTR(4)=ISTAT
310    RETURN
400    RV(1)=RCONC
       CALL MSUB$A(RV,4,1,4,ISTR,8,5,8)
       CALL JSTR$A(A$RGHT,ISTR,8)
       RETURN
       END

======================================================================
```